FORM PTO-1390 (Modified) (REV 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	U.S APPLICATION NO. (IF KNOWN, SEE 37 CFR
20-02	not assigned 09/069799

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/AU00/01048	31 August 2000	31 August 1999

TITLE OF INVENTION
Vaccine Antigens of Moraxella

APPLICANT(S) FOR DO/EO/US
FARN, Jacinta, STRUGNELL, Richard and TENNENT, Jan

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include itens (5), (6), (9) and (24) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☒ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☒ Certificate of Mailing by Express Mail
23. ☐ Other items or information:

JC19 Rec'd PCT/PTO 28 FEB 2002

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
10/069799 not assigned	PCT/AU00/01048	20-02

24. The following fees are submitted:.		CALCULATIONS PTO USE ONLY	
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :			
☒ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO	**$1040.00**		
☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO	**$890.00**		
☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	**$740.00**		
☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)	**$710.00**		
☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)	**$100.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =		**$1,040.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).		**$130.00**	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	46 - 20 =	26	x $18.00	**$468.00**	
Independent claims	17 - 3 =	14	x $84.00	**$1,176.00**	
Multiple Dependent Claims **(check if applicable)**.			☐	**$0.00**	
TOTAL OF ABOVE CALCULATIONS =				**$2,814.00**	
☐ Applicant claims small entity status. See 37 CFR 1.27). The fees indicated above are reduced by 1/2.				**$0.00**	
SUBTOTAL =				**$2,814.00**	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)).			+	**$0.00**	
TOTAL NATIONAL FEE =				**$2,814.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).**			☐	**$0.00**	
TOTAL FEES ENCLOSED =				**$2,814.00**	
				Amount to be: refunded	$
				charged	$

a. ☒ A check in the amount of **$2,814.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **07-1969** A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Greenlee, Winner and Sullivan, P.C.
5370 Manhattan Circle, Suite 201
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Heeja Yoo-Warren
SIGNATURE

Heeja Yoo-Warren
NAME

45,495
REGISTRATION NUMBER

February 28, 2002
DATE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:		:	
	Farn et al.	:	Group Art Unit: not assigned
Serial No.:	10/069,799	:	Examiner: not Assigned
Filed:	February 28, 2002	:	
For:	VACCINE ANTIGENS OF MORAXELLA	:	

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage for Express Mail in an envelope addressed to: The Assistant Commissioner for Patents, BOX PCT, Washington DC 20231

March 29, 2002 — Nina Reel
Date — Nina Reel

EL 936709140 US
Express Mail Tracking Number

SECOND PRELIMINARY AMENDMENT

Asst. Commissioner for Patents
Box PCT
Washington, D.C. 20231

Sir:

Please amend the above-referenced application as follows:

In the specification:

Please insert the following as the first paragraph of the specification:

--CROSS REFERENCE TO RELATED APPLICATIONS

This application is a National Stage Application of PCT International Application No. PCT/AU00/01048, filed on August 31, 2000 which claims priority to Australian Patent Application No. PQ2571, filed on August 31, 1999, both of which are incorporated herein, by reference, in their entirety.--

REMARKS

The application has been amended to claim priority to related patents under 37 C.F.R. 1.78.

CONCLUSION

It is believed that no fee is due with the submission of this Second Preliminary Amendment. However, if this is incorrect, please charge the required fee to Deposit Account No. 07-1969.

Respectfully submitted,

Heeja Yoo-Warren, Ph.D.
Reg. No. 45,495

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Attorney docket No. 20-02
nnr: March 29, 2002

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	:		
Farn et al.	:	Group Art Unit:	not assigned
Serial No.: not assigned	:	Examiner:	not Assigned
Filed: February 27, 2002	:		
For: VACCINE ANTIGENS OF MORAXELLA			

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage for Express Mail in an envelope addressed to: The Assistant Commissioner for Patents, BOX PCT, Washington DC 20231

February 28, 2002 — Date

Nina Reel

EL 571469657 US — Express Mail Tracking Number

PRELIMINARY AMENDMENT

Asst. Commissioner for Patents
Box PCT
Washington, D.C. 20231

Sir:

This amendment accompanies a filing of a U.S. National Stage application under 35 U.S.C. 371. Filing fees for the National Stage application have been calculated based on this amendment.

Please amend the application as follows:

In the Claims:

Please cancel claims 1-38 and enter the following new claims 39-84.

--39. A polypeptide having an amino acid sequence as set out in SEQ ID NO:1 from amino acid 37 to 1114, or a sequence having at least 50% identity thereto, or a functional fragment thereof.

40. The polypeptide, as claimed in claim 39, having a sequence of at least 70% identity with the sequence shown in SEQ ID NO:1 from amino acid 37 to 1114.

41. The polypeptide, as claimed in claim 39, having a sequence of at least 80% identity with the sequence shown in SEQ ID NO:1 from amino acid 37 to 1114.

42. The polypeptide, as claimed in claim 39, having a sequence of at least 90% identity with the sequence shown in SEQ ID NO:1 from amino acid 37 to 1114.

43. A polypeptide, as claimed in claim 39, having protease activity.

44. A nucleic acid molecule comprising a sequence encoding a polypeptide having an amino acid sequence as set out in SEQ ID NO:1 from amino acid 37 to 1114, or a sequence having at least 50% identity thereto, or a functional fragment thereof.

45. A nucleic acid molecule comprising a sequence as set out in SEQ ID NO:2 or a sequence having at least 60% identity thereto, or a sequence which hybridises thereto under stringent conditions.

46. The nucleic acid molecule, as claimed in claim 45, comprising a sequence having at least 70% identity with the sequence shown in SEQ ID NO:2.

47. The nucleic acid molecule, as claimed in claim 45, comprising a sequence having at least 80% identity with the sequence shown in SEQ ID NO:2

48. The nucleic acid molecule, as claimed in claim 45, comprising a sequence having at least 90% identity with the sequence shown in SEQ ID NO:2.

49. A composition for use in raising an immune response in an animal comprising a polypeptide having an amino acid sequence as set out in SEQ ID NO:1 from amino acid 37 to 1114, or a sequence having at least 50% identity thereto, or a functional fragment thereof and optionally a carrier and/or adjuvant.

50. A composition for use in raising an immune response in an animal, comprising a nucleic acid sequence encoding a polypeptide having an amino acid sequence as set out in SEQ ID NO:1 from amino acid 37 to 1114, or a sequence having at least

50% identity thereto, or a functional fragment thereof and optionally a carrier and/or adjuvant.

51. A polypeptide having an amino acid sequence as set out in SEQ ID NO:3 from amino acid 26 to 616, or a sequence having at least 50% identity thereto, or a functional fragment thereof.

52. The polypeptide, as claimed in claim 51, having a sequence of at least 70% identity with the sequence shown in SEQ ID NO:3 from amino acid 26 to 616.

53. The polypeptide, as claimed in claim 51, having a sequence of at least 80% identity with the sequence shown in SEQ ID NO:3 from amino acid 26 to 616.

54. The polypeptide, as claimed in claim 51, having a sequence of at least 90% identity with the sequence shown in SEQ ID NO:3 from amino acid 26 to 616.

55. The polypeptide, as claimed in claim 51, having lipase activity.

56. A nucleic acid molecule comprising a sequence encoding a polypeptide having an amino acid sequence as set out in SEQ ID NO:3 from amino acid 26 to 616, or a sequence having at least 50% identity thereto, or a functional fragment thereof.

57. A nucleic acid molecule comprising a sequence as set out in SEQ ID NO:4 or a sequence having at least 60% identity thereto, or a sequence which hybridises thereto under stringent conditions.

58. The nucleic acid molecule, as claimed in claim 57, comprising a sequence having at least 70% identity with the sequence shown in SEQ ID NO:4.

59. The nucleic acid molecule, as claimed in claim 57, comprising a sequence having at least 80% identity with the sequence shown in SEQ ID NO:4.

60. The nucleic acid molecule, as claimed in claim 57, comprising a sequence having at least 90% identity with the sequence shown in SEQ ID NO:4.

61. A composition for use in raising an immune response in an animal, the composition comprising a polypeptide having an amino acid sequence as set out in SEQ ID NO:3 from amino acid 26 to 616, or a sequence having at least 50% identity thereto, or a functional fragment thereof and optionally a carrier and/or adjuvant.

62. A composition for use in raising an immune response in an animal, the composition comprising a nucleic acid sequence encoding a polypeptide having an amino acid sequence as set out in SEQ ID NO:3 from amino acid 26 to 616, or a sequence having at least 50% identity thereto, or a functional fragment thereof and optionally a carrier and/or adjuvant.

63. A polypeptide having an amino acid sequence as set out in SEQ ID NO:5, or a sequence having at least 60% identity thereto, or a functional fragment thereof.

64. The polypeptide, as claimed in claim 63, having a sequence of at least 70% identity with the sequence shown in SEQ ID NO:5.

65. The polypeptide, as claimed in claim 63, having a sequence of at least 90% identity with the sequence shown in SEQ ID NO:5.

66. The polypeptide, as claimed in claim 63, having haemolysin activity.

67. A nucleic acid molecule comprising a sequence encoding a polypeptide having an amino acid sequence as set out in SEQ ID NO:5, or a sequence having at least 60% identity thereto, or a functional fragment thereof.

68. A nucleic acid molecule comprising a sequence as set out in SEQ ID NO:6 or a sequence having at least 60% identity thereto, or a sequence which hybridises thereto under stringent conditions.

69. The nucleic acid molecule, as claimed in claim 68, comprising a sequence having at least 70% identity with the sequence shown in SEQ ID NO:6.

70. The nucleic acid molecule, as claimed in claim 68, comprising a sequence having at least 80% identity with the sequence shown in SEQ ID NO:6.

71. The nucleic acid molecule, as claimed in claim 68, comprising a sequence having at least 90% identity with the sequence shown in SEQ ID NO:6.

72. A composition for use in raising an immune response in an animal, the composition comprising a polypeptide having an amino acid sequence as set out in SEQ ID NO:5, or a sequence having at least 60% identity thereto, or a functional fragment thereof and optionally a carrier and/or adjuvant.

73. A composition for use in raising an immune response in an animal, the composition comprising a nucleic acid sequence encoding a polypeptide having an amino acid sequence as set out in SEQ ID NO:5, or a sequence having at least 60% identity thereto, or a functional fragment thereof and optionally a carrier and/or adjuvant.

74. A composition for use in raising an immune response in an animal directed against *Moraxella*, the composition comprising at least one polypeptide selected from the group consisting of polypeptide as given in SEQ ID NO:1 from amino acid 37 to 1114, SEQ ID NO:3 from amino acid 26 to 616, and SEQ ID NO:5.

75. The composition of claim 74 wherein said peptide having at least 50% identity to the peptide selected from the group consisting of a peptide as given in SEQ ID NO:1 from amino acid 37 to 1114, SEQ ID NO:3 from amino acid 26 to 616, and SEQ ID NO:5.

76. The composition, as claimed in claim 74, the composition comprising a polypeptide having at least 60% identity to the amino acid sequence as given in SEQ ID NO:5 or a functional fragment thereof, and either one of, or preferably both of, a polypeptide having at least 50% identity to the amino acid sequence as given in SEQ ID NO:1 or a functional fragment thereof and a polypeptide having at least 50% identity to the amino acid sequence as given in SEQ ID NO:3 or a functional fragment thereof.

77. The composition, as claimed in claim 74, wherein the *Moraxella* is *M. bovis* or *M. catarrhalis*.

78. The composition, as claimed in claim 75, wherein the *Moraxella* is *M. bovis* or *M. catarrhalis*.

79. The composition, as claimed in claim 76, wherein the *Moraxella* is *M. bovis* or *M. catarrhalis*.

80. The composition, as claimed in claim 74, wherein the *Moraxella* is *M. bovis*.

81. The composition, as claimed in claim 75, wherein the *Moraxella* is *M. bovis*.

82. The composition, as claimed in claim 76, wherein the *Moraxella* is *M. bovis*.

83. An antibody raised against a polypeptide selected from the group consisting of a polypeptide as given in SEQ ID NO:1 from amino acid 37 to 1114 or a functional fragment thereof, SEQ ID NO:3 from amino acid 26 to 616 or a functional fragment thereof, and SEQ ID NO:5 or a functional fragment thereof.

84. The antibody of claim 83 wherein said polypeptide has at least 50% identity to the polypeptide as given in SEQ ID NO:1 from amino acid 37 to 1114 or a functional fragment thereof, SEQ ID NO:3 from amino acid 26 to 616 or a functional fragment thereof, and SEQ ID NO:5 or a functional fragment thereof.--

REMARKS

The claims of the international application have been replaced to correct formalities. This amendment does not add new matter to the specification.

CONCLUSION

It is believed that no fee is due with the submission of this Preliminary Amendment. However, if this is incorrect, please charge the required fee to Deposit Account No. 07-1969.

Respectfully submitted,

Heeja Yoo-Warren, Ph.D.
Reg. No. 45,495

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Attorney docket No. 20-02
nnr: February 28, 2002

Vaccine antigens of Moraxella

FIELD OF THE INVENTION

The present invention relates to antigens of *Moraxella*, in particular, *Moraxella bovis*, nucleic acid sequences encoding these antigens and formulations for use in raising an immune response against *Moraxella*.

BACKGROUND OF THE INVENTION

Infectious bovine keratoconjunctivitis (IBK) is an economically important disease of cattle caused by the Gram-negative coccobacillus *Moraxella bovis*. More commonly known as pinkeye, IBK is a highly contagious ocular infection which may range from mild conjunctivitis to severe ulceration, corneal perforation and blindness. Therapeutic and preventative measures have limited success in controlling IBK and a vaccine which will prevent the disease is required. A number of factors contribute to the virulence of the organism, the two most important attributes so far identified are the expression of pili, and the ability to produce haemolysin. Seven different serogroups of *M. bovis* strains isolated in Australia, Great Britain and the USA have been characterised, based on pilus types (1). An efficacious pilus-based vaccine must contain a sufficient quantity of pili from all seven serotypes to be fully protective, because of a lack of cross protection between serotypes (2, 3). Expression of all seven pilus serotypes at levels high enough to be useful for commercial vaccine preparation has not been achieved.

The ideal vaccine candidate to stimulate protection against *M. bovis* would be a molecule that is highly-conserved among all strains of this species. Possible candidates are haemolysin, protease, lipase and/or phospholipase (4) enzymes produced by *M. bovis*. For example, a partially purified cell-free supernatant from one haemolytic strain of *M. bovis* has been shown to confer significant protection against heterologous, wild-type challenge (5). The possibility that a haemolysin could be conserved across all seven serotypes of *M. bovis* makes it a potential vaccine candidate against IBK. However, researchers have so far been unable to either clone the gene encoding the haemolysin or purify the protein to homogeneity. Nevertheless, any or all of these molecules, alone or in combination, could prove useful for the generation of an effective vaccine against IBK.

SUMMARY OF THE INVENTION

In a first aspect the present invention consists in a polypeptide, the polypeptide having an amino acid sequence as set out in SEQ. ID. NO. 1 from amino acid 37 to 1114, or a sequence having at least 50% identity thereto, or a functional fragment thereof.

In a preferred embodiment the polypeptide has a sequence of at least 70%, more preferably at least 80% and most preferably at least 90% identity with the sequence shown in SEQ. ID. NO. 1.

In a further preferred embodiment of the first aspect of the present invention the polypeptide has protease activity.

In a second aspect the present invention consists in a nucleic acid molecule, the nucleic acid molecule encoding the polypeptide of the first aspect of the present invention.

In a third aspect the present invention consists in a nucleic acid molecule comprising a sequence as set out in SEQ. ID. NO. 2 or a sequence having at least 60% identity thereto, or a sequence which hybridises thereto under stringent conditions.

In a preferred embodiment the nucleic acid molecule has a sequence of at least 70%, more preferably at least 80% and most preferably at least 90% identity with the sequence shown in SEQ. ID. NO. 2.

In a fourth aspect the present invention consists in a composition for use in raising an immune response in an animal, the composition comprising the polypeptide of the first aspect of the present invention or the nucleic acid sequence of the second aspect of the present invention and optionally a carrier and/or adjuvant.

In a fifth aspect the present invention consists in a polypeptide, the polypeptide having an amino acid sequence as set out in SEQ. ID. NO. 3 from amino acid 26 to 616, or a sequence having at least 50% identity thereto, or a functional fragment thereof.

In a preferred embodiment the polypeptide has a sequence of at least 70%, more preferably at least 80% and most preferably at least 90% identity with the sequence shown in SEQ. ID. NO. 3 from amino acid 26 to 616.

In a further preferred embodiment of the fifth aspect the polypeptide has lipase activity.

In a sixth aspect the present invention consists in a nucleic acid molecule, the nucleic acid molecule encoding the polypeptide of the fifth aspect of the present invention.

In a seventh aspect the present invention consists in a nucleic acid molecule comprising a sequence as set out in SEQ. ID. NO. 4 or a sequence having at least 60% identity thereto, or a sequence which hybridises thereto under stringent conditions.

In a preferred embodiment the nucleic acid molecule has a sequence of at least 70%, more preferably at least 80% and most preferably at least 90% identity with the sequence shown in SEQ. ID. NO. 4.

In an eighth aspect the present invention consists in a composition for use in raising an immune response in an animal, the composition comprising the polypeptide of the fifth aspect of the present invention or the nucleic acid sequence of the sixth aspect of the present invention and optionally a carrier and/or adjuvant.

In a ninth aspect the present invention consists in a polypeptide, the polypeptide having an amino acid sequence as set out in SEQ. ID. NO. 5, or a sequence having at least 60% identity thereto, or a functional fragment thereof.

In a preferred embodiment the polypeptide has a sequence of at least 70%, more preferably at least 80% and most preferably at least 90% identity with the sequence shown in SEQ. ID. NO. 5.

In a further preferred embodiment of the ninth aspect the polypeptide has haemolysin activity.

In a tenth aspect the present invention consists in a nucleic acid molecule, the nucleic acid molecule encoding the polypeptide of the ninth aspect of the present invention.

In an eleventh aspect the present invention consists in a nucleic acid molecule comprising a sequence as set out in SEQ. ID. NO. 6 or a sequence having at least 60% identity thereto, or a sequence which hybridises thereto under stringent conditions.

In a preferred embodiment the nucleic acid molecule has a sequence of at least 70%, more preferably at least 80% and most preferably at least 90% identity with the sequence shown in SEQ. ID. NO. 6.

In a twelfth aspect the present invention consists in a composition for use in raising an immune response in an animal, the composition comprising

the polypeptide of the ninth aspect of the present invention or the nucleic acid sequence of the tenth aspect of the present invention and optionally a carrier and/or adjuvant.

The term "functional fragment" as used herein is intended to cover fragments of the polypeptide which retain at least 10% of the biological activity of the complete polypeptide. In particular this term is used to encompass fragments which show immunological cross-reactivity with the entire polypeptide, eg ligands which react with the fragment also react with the complete polypeptide.

In a thirteenth aspect the present invention consists in a composition for use in raising an immune response in an animal directed against *Moraxella*, the composition comprising at least one polypeptide selected from the group consisting of the polypeptides of the first, fifth and ninth aspects of the present invention and optionally including an adjuvant or carrier.

In a preferred embodiment the composition includes the polypeptide of the ninth aspect of the present invention and either one of, or preferably both, the polypeptides of the first and fifth aspects of the present invention.

In a preferred embodiment the *Moraxella* is *M. bovis or M. catarrhalis,* most preferably *M. bovis*.

In a fourteenth aspect the present invention consists in an antibody raised against a polypeptide selected from the group consisting of the polypeptides of the first, fifth and ninth aspects.

As will be readily appreciated by the person skilled in this field the polypeptides and antibodies of the present invention and probes derived from the nucleotide sequences can be used as diagnostic reagents in determining *Moraxella,* in particular, *M. bovis* infection. For example, the polypeptides and antibodies can be used in ELISA based assays whilst the probes can be used in PCR based assays. The probes will be of a length to provide the required level of specificity and will typically be at least 18 nucleotides in length.

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: Nucleotide and amino acid sequence of a protease from *M. bovis* Dalton 2d. A putative promoter sequence is singly underlined. A putative ribosome binding site is shown in bold and underlined. A putative start codon is shown in bold. Putative transcription terminator sequences are indicated by inverted arrows. Numbering for both the nucleotide and amino acid sequences are shown on the left hand side.

Figure 2: Nucleotide and amino acid sequence of a lipase from *M. bovis* Dalton 2d. A putative promoter sequence is singly underlined. A putative ribosome binding site is shown in bold and underlined. A putative start codon is shown in bold. Putative transcription terminator sequences are indicated by inverted arrows. Numbering for both the nucleotide and amino acid sequences are shown on the left hand side.

Figure 3: Heat stability of the lipase from *M.bovis* when expressed in its recombinant form (pMB1/MC1061). (Heating carried out at 90°C).

Figure 4: Comparison of growth rate and expression levels of the lipase of *M.bovis* when in its (i) native form and (ii) recombinant form. The growth rate is shown as solid bars and the lipase expression levels as open diamonds.

Figure 5: Nucleotide and amino acid sequence of the A subunit of the RTX toxin from *M. bovis* Dalton 2d. A putative ribosome binding site is shown in bold and underlined. A putative start codon is shown in bold. Upstream of the A subunit open reading frame is a portion of the coding region for the C subunit (nucleotide 1 to 195) (corresponding amino acid sequence shown in SEQ ID NO:8) and downstream of the A subunit is a small portion of the B subunit coding region (nucleotide 3080 to 3250) (corresponding amino acid sequence shown in SEQ ID NO:9) . Numbering for both the nucleotide and amino acid sequences are shown on the left hand side.

DETAILED DESCRIPTION OF THE INVENTION

In order that the nature of the present invention may be more clearly understood preferred forms thereof will now be described with reference to the following non-limiting Examples.

General Molecular Biology

Unless otherwise indicated, the recombinant DNA techniques utilized in the present invention are standard procedures, well known to those skilled in the art. Such techniques are described and explained throughout the literature in sources such as, J. Perbal, A Practical Guide to Molecular Cloning, John Wiley and Sons (1984), J. Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbour Laboratory Press (1989), T.A. Brown (editor), Essential Molecular Biology: A Practical Approach, Volumes 1 and 2, IRL Press (1991), D.M. Glover and B.D. Hames (editors), DNA Cloning: A Practical Approach, Volumes 1-4, IRL Press (1995 and 1996), and F.M. Ausubel et al. (Editors), Current Protocols in Molecular Biology, Greene Pub. Associates and Wiley-Interscience (1988, including all updates until present) and are incorporated herein by reference.

Protein Variants

Amino acid sequence variants can be prepared by introducing appropriate nucleotide changes into DNA, or by *in vitro* synthesis of the desired polypeptide. Such variants include, for example, deletions, insertions or substitutions of residues within the amino acid sequence. A combination of deletion, insertion and substitution can be made to arrive at the final construct, provided that the final protein product possesses the desired characteristics. The amino acid changes also may alter post-translational processes such as altering the membrane anchoring characteristics, altering the intra-cellular location by inserting, deleting or otherwise affecting the transmembrane sequences of the native protein, or modifying its susceptibility to proteolytic cleavage.

In designing amino acid sequence variants, the location of the mutation site and the nature of the mutation will depend on characteristic(s) to be modified. The sites for mutation can be modified individually or in

series, e.g., by (1) substituting first with conservative amino acid choices and then with more radical selections depending upon the results achieved, (2) deleting the target residue, or (3) inserting residues of other ligands adjacent to the located site.

A useful method for identification of residues or regions for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (*Science* (1989) **244**: 1081-1085*)*. Here, a residue or group of target residues are identified (e.g., charged residues such as Arg. Asp, His, Lys, and Glu) and replaced by a neutral or negatively charged amino acid (most preferably alanine or polyalanine) to affect the interaction of the amino acids with the surrounding aqueous environment in or outside the cell. Those domains demonstrating functional sensitivity to the substitutions then are refined by introducing further or other variants. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation *per se* need not be predetermined. For example, to optimise the performance of a mutation at a given site, alanine scanning or random mutagenesis may be conducted at the target codon or region and the expressed variants are screened for the optimal combination of desired activity.

There are two principal variables in the construction of amino acid sequence variants; the location of the mutation site and the nature of the mutation. These may represent naturally occurring alleles or predetermined mutant forms made by mutating the DNA either to arrive at an allele or a variant not found in nature. In general, the location and nature of the mutation chosen will depend upon the characteristic to be modified.

Amino acid sequence deletions generally range from about 1 to 30 residues, more preferably about 1 to 10 residues and typically about 1 to 5 contiguous residues.

Amino acid sequence insertions include amino and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Other insertional variants include the fusion of the N- or C-terminus of the proteins to an immunogenic polypeptide e.g. bacterial polypeptides such as betalactamase or an enzyme encoded by the *E. coli trp* locus, or yeast protein, bovine serum albumin, and chemotactic polypeptides. C-terminal fusions with proteins having a

long half-life such as immunoglobulin constant regions (or other immunoglobulin regions), albumin, or ferritin, are included.

Another group of variants are amino acid substitution variants. These variants have at least one amino acid residue in the protein molecule removed and a different residue inserted in its place. The sites of greatest interest for substitutional mutagenesis include sites identified as the active site(s). Other sites of interest are those in which particular residues obtained from various species are identical. These positions may be important for biological activity. These sites, especially those falling within a sequence of at least three other identically conserved sites, are substituted in a relatively conservative manner. Such conservative substitutions are shown in Table 1 under the heading of "preferred substitutions". If such substitutions result in a change in biological activity, then more substantial changes, denominated exemplary substitutions in Table 1, or as further described below in reference to amino acid classes, are introduced and the products screened.

TABLE 1

Original Residue	**Exemplary Substitutions**	**Preferred Substitutions**
Ala (A)	val; leu; ile	val
Arg (R)	lys; gln; asn	lys
Asn (N)	gln; his; lys; arg	gln
Asp (D)	glu	glu
Cys (C)	ser	ser
Gln (Q)	asn	asn
Glu (E)	asp	asp
Gly (G)	pro	pro
His (H)	asn; gln; lys; arg	arg
Ile (I)	leu; val; met; ala; phe norleucine	leu
Leu (L)	norleucine, ile; val; met; ala; phe	ile

Original Residue	Exemplary Substitutions	Preferred Substitutions
Lys (K)	arg; gln; asn	arg
Met (M)	leu; phe; ile;	leu
Phe (F)	leu; val; ile; ala	leu
Pro (P)	gly	gly
Ser (S)	thr	thr
Thr (T	ser	ser
Trp (W)	tyr	tyr
Tyr (Y)	trp; phe; thr; ser	phe
Val (V)	ile; leu; met; phe; ala; norleucine	leu

Mutants, Variants and Homology - Proteins

Mutant polypeptides will possess one or more mutations which are deletions, insertions, or substitutions of amino acid residues. Mutants can be either naturally occurring (that is to say, purified or isolated from a natural source) or synthetic (for example, by performing site-directed mutagensis on the encoding DNA). It is thus apparent that polypeptides of the invention can be either naturally occurring or recombinant (that is to say prepared using recombinant DNA techniques).

An allelic variant will be a variant that is naturally occurring within an individual organism.

Protein sequences are homologous if they are related by divergence from a common ancestor. Consequently, a species homologue of the protein will be the equivalent protein which occurs naturally in another species. Within any one species a homologue may exist as numerous allelic variants, and these will be considered homologues of the protein. Allelic variants and species homologues can be obtained by following standard techniques known to those skilled in the art. Preferred species homologues include those obtained from representatives of the same Phylum, more preferably the same Class and even more preferably the same Order.

A protein at least 50% identical, as determined by methods well known to those skilled in the art (for example, the method described by Smith, T.F. and Waterman, M.S. (1981) Ad. Appl Math., 2: 482-489, or Needleman, S.B. and Wunsch, C.D. (1970) J. Mol. Biol., 48: 443-453), to that of the present invention are included in the invention, as are proteins at least 70% or 80% and more preferably at least 90% identical to the protein of the present invention. This will generally be over a region of at least 20, preferably at least 30, contiguous amino acids.

Mutants, Variants and Homology - Nucleic Acids

Mutant polynucleotides will possess one or more mutations which are deletions, insertions, or substitutions of nucleotide residues. Mutants can be either naturally occurring (that is to say, isolated from a natural source) or synthetic (for example, by performing site-directed mutagensis on the DNA). It is thus apparent that polynucleotides of the invention can be either naturally occurring or recombinant (that is to say prepared using recombinant DNA techniques).

An allelic variant will be a variant that is naturally occurring within an individual organism.

Nucleotide sequences are homologous if they are related by divergence from a common ancestor. Consequently, a species homologue of the polynucleotide will be the equivalent polynucleotide which occurs naturally in another species. Within any one species a homologue may exist as numerous allelic variants, and these will be considered homologues of the polynucleotide. Allelic variants and species homologues can be obtained by following standard techniques known to those skilled in the art. Preferred species homologues include those obtained from representatives of the same Phylum, more preferably the same Class and even more preferably the same Order.

A polynucleotide at least 70% identical, as determined by methods well known to those skilled in the art (for example, the method described by Smith, T.F. and Waterman, M.S. (1981) Ad. Appl Math., 2: 482-489, or Needleman, S.B. and Wunsch, C.D. (1970) J. Mol. Biol., 48: 443-453), to that of the present invention are included in the invention, as are proteins at least 80% or 90% and more preferably at least 95% identical to the

polynucleotide of the present invention. This will generally be over a region of at least 60, preferably at least 90, contiguous nucleotide residues.

Antibody Production

The term "antibody" should be construed as covering any specific binding substance having a binding domain with the required specificity. Thus, the term covers antibody fragments, derivatives, functional equivalents and homologues of antibodies, including any polypeptide including an immunoglobulin binding domain, whether natural or synthetic. Chimaeric molecules including an immunoglobulin binding domain, or equivalent, fused to another polypeptide are therefore included.

Antibodies, either polyclonal or monoclonal, which are specific for a protein of the present invention can be produced by a person skilled in the art using standard techniques such as, but not limited to, those described by Harlow et al. Antibodies: A Laboratory Manual, Cold Springs Harbor Laboratory Press (1988), and D. Catty (editor), Antibodies: A Practical Approach, IRL Press (1988).

Various procedures known in the art may be used for the production of polyclonal antibodies to epitopes of a protein. For the production of polyclonal antibodies, a number of host animals are acceptable for the generation of antibodies by immunization with one or more injections of a polypeptide preparation, including but not limited to rabbits, mice, rats, etc. Various adjuvants may be used to increase the immunological response in the host animal, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels such as aluminium hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, oil emulsions, keyhole lympet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum.

A monoclonal antibody to an epitope of a protein may be prepared by using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include but are not limited to the hybridoma technique originally described by Kohler and Milstein (1975, Nature 256, 493-497), and the more recent human B-cell hybridoma technique (Kesber *et al.* 1983, Immunology Today 4:72) and EBV-hybridoma technique (Cole *et al.* 1985, Monoclonal Antibodies and

Cancer Therapy, Alan R. Liss, Inc. pp. 77-96). In addition, techniques developed for the production of "chimeric antibodies" by splicing the genes from an antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity may be used (Morrison *et al.* 1984, Proc. Natl. Acad. Sci., 81:6851-6855; Neuberger *et al.* 1984 Nature 312:604-608; Takeda *et al.* 1985 Nature 31:452-454). Alternatively, techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce 4-specific single chain antibodies.

Recombinant human or humanized versions of monoclonal antibodies are a preferred embodiment for human therapeutic applications. Humanized antibodies may be prepared according to procedures in the literature (e.g. Jones *et al.* 1986, Nature 321:522-25; Reichman *et al.* 1988, Nature 332:323-27; Verhoeyen *et al.* 1988, Science 239:1534-36). The recently described "gene conversion mutagenesis" strategy for the production of humanized monoclonal antibody may also be employed in the production of humanized antibodies (Carter *et al.* 1992 Proc. Natl. Acad. Sci. U.S.A. 89:4285-89). Alternatively, techniques for generating the recombinant phage library of random combinations of heavy and light regions may be used to prepare recombinant antibodies (e.g. Huse *et al.* 1989 Science 246:1275-81).

Antibody fragments which contain the idiotype of the molecule such as Fu $F(ab^1)$ and $F(ab^2)$ may be generated by known techniques. For example, such fragments include but are not limited to: the F(ab) E2 fragment which can be produced by pepsin digestion of the intact antibody molecule; the Fab' fragments which can be generated by reducing the disulfide bridges of the F(ab')2 fragment, and the two Fab fragments which can be generated by treating the antibody molecule with papain and a reducing agent. Alternatively, Fab expression libraries may be constructed (Huse *et al.* 1989, Science 246:1275-1281) to allow rapid and easy cloning of a monoclonal Fab fragment with the desired specificity to a protein.

Adjuvants and Carriers

Pharmaceutically acceptable carriers or diluents include those used in compositions suitable for oral, rectal, nasal, topical (including buccal and sublingual), vaginal, parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intrathecal and epidural) administration. They are

non-toxic to recipients at the dosages and concentrations employed. Representative examples of pharmaceutically acceptable carriers or diluents include, but are not limited to water, isotonic solutions which are preferably buffered at a physiological pH (such as phosphate-buffered saline or Tris-buffered saline) and can also contain one or more of, mannitol, lactose, trehalose, dextrose, glycerol, ethanol or polypeptides (such as human serum albumin). The compositions may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy.

As mentioned above the composition may include an adjuvant. As will be understood an "adjuvant" means a composition comprised of one or more substances that enhances the immunogenicity and efficacy of a vaccine composition. Non-limiting examples of suitable adjuvants include squalane and squalene (or other oils of animal origin); block copolymers; detergents such as Tween®-80; Quil® A, mineral oils such as Drakeol or Marcol, vegetable oils such as peanut oil; *Corynebacterium*-derived adjuvants such as *Corynebacterium parvum*; *Propionibacterium*-derived adjuvants such as *Propionibacterium acne*; *Mycobacterium bovis* (Bacille Calmette and Guerin or BCG); interleukins such as interleukin 2 and interleukin 12; monokines such as interleukin 1; tumour necrosis factor; interferons such as gamma interferon; combinations such as saponin-aluminium hydroxide or Quil-A aluminium hydroxide; liposomes; ISCOM adjuvant; mycobacterial cell wall extract; synthetic glycopeptides such as murarmyl dipeptides or other derivatives; Avridine; Lipid A derivatives; dextran sulfate; DEAE-Dextran or with aluminium phosphate; carboxypolymethylene such as Carbopol' EMA; acrylic copolymer emulsions such as Neocryl A640 (e.g. U.S. Pat. No. 5,047,238); vaccinia or animal poxvirus proteins; sub-viral particle adjuvants such as cholera toxin, or mixtures thereof.

Gene/DNA Isolation

The DNA encoding a protein may be obtained from any cDNA library prepared from tissue believed to express the gene mRNA and to express it at a detectable level. DNA can also be obtained from a genomic library.

Libraries are screened with probes or analytical tools designed to identify the gene of interest or the protein encoded by it. For cDNA expression libraries, suitable probes include monoclonal or polyclonal

antibodies that recognize and specifically bind the protein; oligonucleotides of about 20-80 bases in length that encode known or suspected portions of cDNA from the same or different species; and/or complementary or homologous cDNAs or fragments thereof that encode the same or a hybridizing gene. Appropriate probes for screening genomic DNA libraries include, but are not limited to, oligonucleotides; cDNAs or fragments thereof that encode the same or hybridizing DNA including expressed sequence tags and the like; and/or homologous genomic DNAs or fragments thereof. Screening the cDNA or genomic library with the selected probe may be conducted using standard procedures as described in chapters 10-12 of Sambrook *et al.*

An alternative means to isolate a gene encoding the protein of interest is to use polymerase chain reaction (PCR) methodology as described in section 14 of Sambrook *et al.* This method requires the use of oligonucleotide probes that will hybridize to the gene.

The oligonucleotide sequences selected as probes should be of sufficient length and sufficiently unambiguous that false positives are minimized. The actual nucleotide sequence(s) is usually based on conserved or highly homologous nucleotide sequences or regions of the gene. The oligonucleotides may be degenerate at one or more positions. The use of degenerate oligonucleotides may be of particular importance where a library is screened from a species in which preferential codon usage in that species is known. The oligonucleotide must be labelled such that it can be detected upon hybridization to DNA in the library being screened. The preferred method of labelling is to use (α-^{32}P)- dATP with polynucleotide kinase, as is well known in the art, to radiolabel the oligonucleotide. However, other methods may be used to label the oligonucleotide, including, but not limited to, biotinylation or enzyme labelling.

DNA encompassing all the protein coding sequence is obtained by screening selected cDNA or genomic libraries, and if necessary, using conventional primer extension procedures as described in section 7.79 of Sambrook *et al.*, to detect precursors and processing intermediates of mRNA that may not have been reverse-transcribed into cDNA.

Another alternative method for obtaining the gene of interest is to chemically synthesize it using one of the methods described in Fingels *et al.* (*Agnew Chem. Int. Ed. Engl. 28:* 716-734, 1989). These methods include

triester, phosphite, phosphoramidite and H-Phosphonate methods, PCR and other autoprimer methods, and oligonucleotide syntheses on solid supports. These methods may be used if the entire nucleic acid sequence of the gene is known, or the sequence of the nucleic acid complementary to the coding strand is available, or alternatively, if the target amino acid sequence is known, one may infer potential nucleic acid sequences using known and preferred coding residues for each amino acid residue.

Substantially Purified

By "substantially purified" we mean a polypeptide that has been separated from lipids, nucleic acids, other polypeptides, and other contaminating molecules.

Hybridisation

The polynucleotide sequence of the present invention may hybridise to the respective sequence set out SEQ. ID. NOS. 2, 4, or 6 under high stringency. As used herein, stringent conditions are those that (i) employ low ionic strength and high temperature for washing after hybridization, for example, 0.1 x SSC and 0.1% (w/v) SDS at 50°C; (ii) employ during hybridization conditions such that the hybridization temperature is 25°C lower than the duplex melting temperature of the hybridizing polynucleotides, for example 1.5 x SSPE, 10% (w/v) polyethylene glycol 6000, 7% (w/v) SDS, 0.25 mg/ml fragmented herring sperm DNA at 65°C; or (iii) for example, 0.5M sodium phosphate, pH 7.2, 5mM EDTA, 7% (w/v) SDS and 0.5% (w/v) BLOTTO at 70°C; or (iv) employ during hybridization a denaturing agent such as formamide, for example, 50% (v/v) formamide with 5 x SSC, 50mM sodium phosphate (pH 6.5) and 5 x Denhardt's solution (32) at 42°C; or (v) employ, for example, 50% (v/v) formamide, 5 x SSC, 50mM sodium phosphate (pH 6.8), 0.1% (w/v) sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50μg/ml) and 10% dextran sulphate at 42°C.

EXAMPLE 1

This example describes the cloning and characterisation of a protease from *Moraxella bovis*.

Bacteria and construction of a genomic library

Moraxella bovis strain Dalton 2d was a field isolate collected from a bovine eye and characterised by CSIRO Animal Health, Parkville, Australia (6). *Escherichia coli* strain DH5α has been previously described (7, 8).

All enzymes were purchased from Promega (Madison, WI, USA) except where otherwise noted.

General cloning and DNA techniques were as described (9) unless otherwise noted.

A genomic library was constructed by carrying out partial *Sau*3A digests on genomic DNA extracted from *M. bovis* strain Dalton 2d using a CTAB method which is outlined below. This DNA was size fractionated using a NaCl gradient (10) and ligated with the cosmid cloning vector pHC79 (11) which had been previously digested with *Bam*HI. This DNA was packaged into lambda bacteriophage heads using the Packagene Lambda DNA packaging system (Promega, Madison,WI, USA) and this was used to transduce the *E. coli* strain DH5α. The library was stored in 96 well trays (50% glycerol / luria broth / ampicillin (50μg/ml)) at -70°C.

CTAB genomic DNA extraction from *M. bovis*

A 5ml brain heart infusion (BHI) (Oxoid Ltd., Basingstoke, Hampshire, U.K.) broth was inoculated with a colony of Dalton 2d taken from a fresh overnight culture on horse blood agar and incubated with shaking at 37°C for 6 hours. This culture was used to inoculate 50ml of BHI broth which was grown with shaking at 37°C overnight. 40ml of the culture was transferred to an SS34 tube and the cells pelleted at 3000 × g for 10 minutes. Following resuspension of the pellet in 9.5ml of 25% sucrose in TE buffer (10mM Tris, 1mM EDTA (pH8)), 500μl of 10% SDS, 50μl of 20mg/ml proteinase K and 20μl of 10mg/ml RnaseA were added and this mixture incubated in an orbital shaker for 1 hour at 37°C. To this mixture, 1.8ml of 5M NaCl and 1.5ml of a CTAB (N-Cetyl-N,N,N-trimethyl-ammonium bromide) / NaCl solution was added and incubation continued for 20 minutes at 65°C. The DNA was

extracted using phenol/chloroform and precipitated with 0.6 volumes of isopropanol. The resulting DNA was washed in 70% ethanol, dried and resuspended in 2ml of TE buffer.

Screening of genomic library for enzyme activity

The genomic library was cultured on skim milk agar to screen for the presence of a clone displaying protease activity (double strength Columbia agar base (Oxoid Ltd., Basingstoke, Hampshire, U.K.) / 10% skim milk) for 24 hours at 37°C followed by refrigeration at 4°C for one to two days.

A single clone from the genomic library was detected as having activity against skim milk agar. DNA analysis confirmed that the clone contained a fragment of *M. bovis* Dalton 2d genomic DNA approximately 40 kilobases in size. The construct was designated pJF1.

Nucleotide sequence of the *M. bovis* protease clone pJF1

Plasmid and cosmid DNA for automated sequencing was extracted using the Wizard Plus SV Minipreps DNA Purification System (Promega, Madison, WI, USA) and the Qiagen Plasmid Midi Kit (Qiagen Pty. Ltd., Clifton Hill, Vic, Australia), respectively.

The nucleotide sequence of the insert DNA was determined using the process of "primer walking" (12). This was achieved using synthetic oligonucleotides (Bresatec / Geneworks, Thebarton, SA, Australia) and the dye terminator cycle sequencing ready reaction (Perkin Elmer Corporation, Norwalk, CT, USA). The resulting sequence was analysed on an Applied Biosystems 373A DNA sequencer.

Automated sequencing revealed an open reading frame of 3345bp capable of encoding a protein of 1115 amino acids. The sequence is written in the 5' to 3' direction and is shown in Figure 1 together with the corresponding amino acid sequence which is predicted to encode a protein with a molecular weight of 120kDa. The amino acid sequence is shown in SEQ. ID. NO. 1 and the DNA sequence is shown in SEQ. ID. NO. 2.

The putative start codon for the mature protease protein was identified by the presence of a possible ribosome binding site upstream. This RBS was identified by its similarity to the consensus sequence for the *E. coli* RBS and that previously identified for the *M. bovis* pilin genes (AGGAG) (27)

Due to the secreted nature of the protease, it was assumed that it would contain in its N-terminal sequence a signal peptide which would be used in the secretion of the protein. This analysis was carried out using a prediction program (SignalP) available through the Expasy website (http://www.expasy.ch/tools/), which allows for the identification of prokaryotic signal peptides and predicts possible cleavage sites. This analysis only identified a signal peptide using the start codon indicated in the accompanying protein/DNA sequence.

Sequence comparisons

Comparisons of the deduced amino acid sequence with those in the database were carried out using the BlastX and BlastP programs (13) which are available at http://www.ncbi.nlm.nih.gov.

At the amino acid level, the protease cloned from Dalton 2d displayed the following similarity and identity to the proteins listed.

Organism	Protein	Similarity	Identity
Serratia marcescens	ssp-h2 - serine protease autotransporter	39%	23%
Serratia marcescens	ssp-h1 - serine protease autotransporter	37%	22%
Pseudomonas flourescens	serine protease homologue	34%	20%
Pseudomonas tolaasii	serine protease	35%	21%

More generally the 5' domain of the *M. bovis* protease displays homology to a family of subtilisins (serine proteases) while the 3' region resembles a number of outer membrane proteins.

The *M. bovis* sequence was found to contain a highly proline rich region which distinguished it from all other proteins to which it was closely related.

Protease type encoded by pJF1

In order to identify the type of protease activity encoded by pJF1, a range of specific protease inhibitors were examined for their effect on the expression of the *M. bovis* protease.

The method of Bourgeau *et al.*, (1992) (14) was used to determine inhibitor activity with the following modifications. 100µl of cell free supernatant from a fresh overnight broth culture was mixed with 650µl of 100mM Tris (pH 7.2) and a suitable volume of inhibitor PMSF (phenylmethylsulfonyl fluoride) 5mM; EDTA 5mM; leupeptin 100µg/ml; pepstatin 50µg/ml]. Distilled water was used to make the volume up to 1ml. The mixture was incubated at 37°C for 30 minutes and 10mg of azocoll (Calbiochem, Alexandria, NSW, Australia) was then added. The suspensions were incubated at 37°C for 16 hours and the optical density read at 520nm.

In this way it was confirmed that the activity attributable to the protease encoded by pJF1 was that of a serine protease since PMSF (a serine protease inhibitor) reduced the protease activity of both Dalton 2d and pJF1 to zero.

Conservation of protease in *M. bovis*

Southern hybridisation using an internal fragment of the protease coding region as a probe was carried out to investigate whether the protease was present in strains representing the known *M. bovis* pili serotypes.

Genomic DNA extracted from the representative strains of *M. bovis* (15) was digested with *Xba*I and *Eco*RI and separated using agarose gel electrophoresis. The DNA was transferred to a Hybond N+ filter (Amersham, Little Chalfont, Buckinghamshire, U.K.) using the method described (9). The probe used in the southern hybridisation was a PCR amplified fragment which was internal to the protease coding region. This fragment was labelled with α^{32}P-dATP using the Megaprime labelling system (Amersham, Little Chalfont, Buckinghamshire, U.K.) according to the manufacturers instructions. High stringency conditions were used (hybridisation temperature 68°C; 2 washes at room temperature in 2 × SSC / 0.1% SDS; 1 wash at 68°C in 0.1 × SSC / 0.1% SDS) and the resulting filters were exposed to autoradiographic film (Kodak, Rochester, New York, USA) for 5 to 24 hours before developing.

Results showed that the protease gene cloned in pJF1 is present in all strains of *M. bovis* examined.

EXAMPLE 2

This example describes the cloning and characterisation of a lipase from *Moraxella bovis*.

Bacteria and construction of a plasmid library

Moraxella bovis strain Dalton 2d was a field isolate collected from a bovine eye and characterised by CSIRO Animal Health, Parkville, Australia (6). *Escherichia coli* strain MC1061 has been previously described (16).

All enzymes were purchased from Promega (Madison, WI, USA) except where otherwise noted. General cloning and DNA techniques were as described (9) unless otherwise noted.

A plasmid library was constructed in the cloning vector pBR322 (17). This was done by partially digesting genomic DNA extracted from Dalton 2d (using the CTAB method described in Example 1) with *Sau*3A under conditions that maximised the amount of DNA in the range of 1 to 2kb. This DNA was ligated with pBR322 which had been previously digested with *Bam*HI. The ligated DNA was electroporated (2.5kV, 200Ω and 200μF, for a theoretical time constant of 4.7) into electrocompetent *E. coli* MC1061 cells.

Screening of plasmid library for lipase expression

Following electroporation of the ligated DNA into MC1061 cells, recombinant clones displaying lipase activity were detected by culturing the library for 24 hours at 37°C on media containing Tween 80 [10ml Tween 80 (Sigma, St Louis, MO, USA), 5g NaCl, 3g agar No.1 (Oxoid Ltd., Basingstoke, Hampshire, U.K.), 10g peptone, 0.1g $CaCl_2.H_20$ / litre].

Twenty eight out of 24,000 clones screened were found to be displaying lipase activity. DNA analysis confirmed that all of these clones contained one 5.4kb fragment of DNA in common. One clone was chosen to continue work with and this was designated pMB1.

In some experiments (below), a photometric assay of extracellular lipase activity was performed with p-nitrophenylpalmitate as the substrate (18, 19). Strains of *E. coli* and/or *M. bovis* were grown at 37°C for the required time points. Cell free culture supernatant (100μl) was mixed with 2.4ml of enzyme buffer (19) to assay secreted lipase activity. After 30 minutes incubation at 37°C, the optical density at 410nm was determined.

One enzyme unit was defined as the amount of enzyme that releases 1 nmol of p-nitrophenyl from p-nitrophenylpalmitate ml^{-1} min^{-1}. Under the conditions described by Stuer *et al.*, (18), an optical density at 410nm of 0.041 is equivalent to 1 enzyme unit.

Nucleotide sequence of the *M. bovis* lipase clone pMB1

Plasmid pMB1 was subjected to automated DNA sequencing using the methodology described in Example 1.

This analysis revealed an open reading frame of 1851bp capable of encoding 617 amino acids. The sequence is written in the 5' to 3' direction and is shown in Figure 2 together with the corresponding amino acid sequence that is predicted to encode a protein with a molecular weight of 65.8kDa. The amino acid sequence is shown in SEQ. ID. NO. 3 and the DNA sequence is shown in SEQ. ID. NO. 4.

The techniques set out above in respect of the protease were used to identify the potential start codon for the lipase protein.

Sequence comparisons

Sequence comparisons were made using the methodology described in Example 1.

At the amino acid level, the lipase cloned from *M. bovis* Dalton 2d was shown to display the following similarity and identity to the proteins listed.

Organism	Protein	Similarity	Identity
Xenorhabdus luminescens	triacylglycerol lipase	36%	24%
Pseudomonas putida	hypothetical protein	36%	24%
Salmonella typhimurium	outer membrane esterase	35%	23%
Pseudomonas aeruginosa	lipase / esterase	36%	23%

The *M. bovis* lipase was identified as being a possible new member of the GDSL family (20) of lipolytic enzymes.

N-terminal sequencing carried out on the lipase mature protein

The required strains of *E. coli* were cultured overnight with shaking at 37°C in 500mls of luria broth. The cells were pelleted at 5,000 rpm for 15 mins and the supernatant filtered through a 0.45µm filter. Solid ammonium sulfate was added to the supernatant to 60% saturation (180g / 500ml), and dissolved at 4°C with stirring for 30 minutes. This mixture was left at 4°C overnight and the precipitated proteins pelleted at 7,000 rpm for 30 mins. The proteins were resuspended in 3ml of double distilled water and the solubilised proteins dialysed against double distilled water overnight to remove any salt. The resulting mixture was filtered through a 0.45µm filter and stored at -20°C.

Following separation of the proteins by SDS-PAGE, the proteins were transferred to PVDF membrane and excised. The protein was subjected to automated (Edman degradation) sequence analysis (28) with vapour phase delivery of critical reagents (29) in an automated sequenator (model 470A; Applied Biosystems) (Applied Biosystems Division, Foster City, CA, USA) in conjunction with a PTH amino acid separation system (model 120A PTH analyzer; Applied Biosystems).

Using this technique 17 amino acids with two gaps were identified

K E F S Q V I I F G D S L X D X G (SEQ ID NO:7)

which corresponds exactly with amino acids 26 through to 42 shown on the accompanying sequence. This result also indicated that the protein most likely includes an amino terminal signal peptide which is involved in the secretion of the protein. This amino terminal corresponds to amino acids 1 through to 25 in the accompanying sequence.

Raising antibodies to the lipase in rabbits

Antibody to the recombinant lipase was raised in rabbits by injecting ammonium sulfate precipitated supernatant from *E. coli* MC1061/pMB4. Prior to vaccination, the lipase preparation was inactivated by heating to 90°C for 90min. 30µg of this protein was injected at 2 weekly intervals for 4 weeks. The primary inoculum was emulsified with Freunds complete adjuvant and subsequent vaccinations with Freunds incomplete adjuvant.

Heat stability of *M. bovis* lipase

The recombinant lipase cloned from *M. bovis* Dalton 2d was found to be very heat stable since it required heating at 90°C for 105 minutes for the

activity to be reduced by 97%. Figure 3 illustrates this phenomenon with enzyme activity expressed as "lipase enzyme units" as determined in the extracellular lipase assay.

Relative expression levels of native versus recombinant lipase

An experiment was performed to plot growth rate with lipase production and to compare production of the recombinant lipase from MC1061/pMB1 with that of the native form of the lipase from *M. bovis* Dalton 2d. Figure 4 illustrates that the two strains grow at approximately the same rate but they do not reach the same cell density, with Dalton 2d substantially lower after 9 hours than MC1061/pMB1. Lipase expression levels were greatest from the pMB1 construct in *E. coli* compared to native lipase expression from *M. bovis* Dalton 2d.

This result was further substantiated when proteins from cell-free supernatants of either the *E. coli* clone or *M. bovis* Dalton 2d were ammonium sulfate precipitated and analysed by SDS-PAGE and western blot using antisera to the recombinant heat-deactivated lipase.

Ammonium sulfate precipitated supernatants were prepared from overnight cultures of *E. coli* or *M. bovis* that had been shaken at 37°C in either 500mls of Luria broth or brain heart infusion broth, respectively. Cells were pelleted at 5000 × g for 15 minutes and the supernatant filtered through a 0.45µm filter. Solid ammonium sulfate was added to the supernatant to 60% saturation (180g / 500ml) and dissolved at 4°C with stirring for 30 minutes. This mixture was left at 4°C overnight and the precipitated protein pelleted at 7000 × g for 30 minutes. Proteins were resuspended in 3ml of double distilled water and the solubilised proteins dialysed against double distilled water overnight to remove any salt. The resulting mixture was filtered through a 0.45µm filter and stored at -20°C.

Protein samples (100µl) were prepared for SDS-PAGE by resuspension in 100µl of 2x sample buffer (5ml 0.5M Tris (pH6.8), 8ml 10% SDS, 4ml glycerol, 0.8ml β-mercaptoethanol, 1ml double distilled H_2O, bromophenol blue) and heating to 100°C for 5 minutes. The proteins were separated on a 12.5% polyacrylamide gel using the buffer system of Laemlli (21).

Western blots were carried out according to the method of Towbin *et al.*, (22) and following separation of proteins by SDS-PAGE and transfer to nitrocellulose using the Bio-Rad minicell (Bio-Rad, Hercules, CA, USA)

transfer system. Filters were immunoblotted with the recombinant lipase antiserum (at a concentration of 1/100) which had been adsorbed against MC1061 cells. The antiserum was raised against ammonium sulfate precipitated recombinant lipase which had been heat deactivated (1 hour 45 minutes at 90°C) and used to inoculate rabbits (three doses of 50µg each) at 4 week intervals. Blood samples were collected from the marginal ear vein prior to immunisation and at each vaccination time point.

The results showed a prominent band present in the recombinant lipase positive construct MC1061/pMB1 that is detectable in relatively minor amounts in *M. bovis* Dalton 2d preparation. The protein detected with the antisera was approximately the same size as that of the predicted molecular weight for the *M. bovis* lipase (65.8kDa).

Lipase type encoded by pMB1

Thin layer chromatography (TLC) was used to determine whether the lipase of *M. bovis* Dalton 2d displayed phospholipase activity. Characterisation of phospholipase type essentially followed a previously described method (23) except that the results of separation on Silica Gel 60 plates were visualised by developing with a 10% ethanolic solution of molybdophosphoric acid at 100°C. All reagents used were purchased from Sigma (Sigma, St Louis, MO, USA).

TLC determined that the *M. bovis* lipase displayed the same enzyme specificity as that of a commercially-available phospholipase B when lysophosphatidylcholine and phosphatidylcholine were used as substrates (data not shown).

Conservation of lipase among *M. bovis*

A southern blot using an internal fragment of the Dalton 2d lipase coding region was used to investigate whether the lipase gene was present in strains of *M. bovis* representing the known pilus serotypes.

Genomic DNA extracted from the strains of *M. bovis* representing each of the known pilus serotypes (15) was digested with *Hin*dIII and separated using agarose gel electrophoresis. The DNA was transferred to a Hybond N+ filter (Amersham, Little Chalfont, Buckinghamshire, U.K.) using a previously described method (9). The probe used in the southern hybridisation was a *Hin*dIII fragment that contained sequence internal to the lipase coding

region. This fragment was labelled with α^{32}P-dATP using the Megaprime labelling system (Amersham, Little Chalfont, Buckinghamshire, U.K.) according to the manufacturers instructions. High stringency conditions were used (hybridisation temperature 68°C; 2 washes at room temperature in 2 × SSC / 0.1% SDS; 1 wash at 68°C in 0.1 × SSC / 0.1% SDS) and the resulting filters were exposed to autoradiographic film (Kodak, Rochester, New York, USA) for 5 to 24 hours before developing.

Results showed that the lipase gene is present in all strains of *M. bovis* examined.

To confirm whether or not the lipase gene was expressed in each of the serotype representative strains, antisera raised against recombinant heat deactivated lipase was used in a western blot analysis of whole cell preparations. Results showed that the lipase was indeed being expressed by all of these *M. bovis* strains.

EXAMPLE 3

Bacteria and construction of a haemolysin clone

Moraxella bovis strain Dalton 2d was a field isolate collected from a bovine eye and characterised by CSIRO Animal Health, Parkville, Australia (6).

All of the *M. bovis* strains representative of the known pilus serotypes express a haemolytic activity that is detected on horse blood agar.

Escherichia coli strain degP4::Tn5 has a leaky outer membrane and is defective in proteolysis and has been previously described (24).
All enzymes were purchased from Promega (Madison, WI, USA) except where otherwise noted.

General cloning and DNA techniques were as described (9) unless otherwise noted.

A *phoA* fusion technique that allows for the identification of exported proteins (25) was utilised with some modifications. Genomic DNA from *M. bovis* Dalton 2d (prepared using the CTAB method described in Example 1) was partially digested with *Sau*3A. Restricted DNA was ligated with a series of vectors that allow fusions with an alkaline phosphatase gene in three different reading frames. The ligated DNA was electroporated into *E. coli* degP4::Tn5 and the resulting clones screened on Luria agar containing

ampicillin (50μg/ml) and X-P (200μg/ml) (5-bromo-3-chloro-indolyl phosphate). Selection of clones relies on the observation that if the fragment is cloned in frame and contains an export sequence the resulting colony will be blue in colour. The leaky *E. coli* strain allows the outer membrane-bound proteins and secreted proteins (both fused with *phoA*) to be distinguished from non-secreted fusion proteins.

Sequencing of the *M. bovis* haemolysin determinant

Clones selected for the presence of a secreted or outer membrane protein gene sequence were subjected to automated DNA sequencing using the methods described in Example 1. One of these clones, pMbh1, was found to contain 200bp of DNA which displayed high homology to the A subunit of other RTX toxins. Inverse PCR and degenerate oligonucleotides were utilised to obtain the sequence of the entire A subunit. The open reading frame of 2784bp was capable of encoding 928 amino acids. The sequence is written in the 5' to 3' direction and is shown in Figure 5 together with the corresponding amino acid sequence that is predicted to encode a protein with a molecular weight of 98.8kDa. The amino acid sequence is shown in SEQ. ID. NO. 5 and the DNA sequence is shown in SEQ. ID. NO. 6.

The putative start codon was identified using the RBS technique outlined above. A signal peptide analysis was not carried out as the A subunit is not secreted on its own. However as the protein sequence of these proteins (RTX) is quite highly conserved, on amino acid homologies alone this start codon was the one of choice.

Sequence homology

At the amino acid level the *M. bovis* Dalton 2d haemolysin gene product shows striking similarity to the A subunit of the of several RTX and other haemolysins as shown in the following table.

Organism	Protein	Similarity	Identity
Pasteurella haemolytica	LktA protein (leukotoxin)	68%	50%
Actinobacillus pleuropneumoniae	RTX toxin determinant	68%	48%
Escherichia coli	Haemolysin - plasmid	58%	43%
E. coli	Haemolysin - chromosomal	58%	43%

Functional complementation by the *M. bovis* haemolysin

A construct which expressed the chromosomal-borne haemolysin of *E. coli* was obtained (pLG900; generated by combining the two plasmids pLG575 (26) and pLG816 (*hlyC* and *hlyA* cloned into pBluescriptSK). pLG900 comprises the four genes of the RTX operon, *hlyC, hlyA, hlyB, hlyD,* cloned into pBluescriptSK and is capable of conferring a haemolytic phenotype on *E. coli* cells that were previously non-haemolytic. The A subunit (*hlyA*) of this construct was mutated such that it was no longer able to be expressed but the other genes involved in the operon (*hlyB, hlyC* and *hlyD*) remained intact. The *E. coli* strain containing this construct (pLG900 / *hlyA* negative) was no longer haemolytic. However, the haemolytic phenotype was restored by providing *in trans* the cloned haemolysin subunit gene from *M. bovis* Dalton 2d. Thus it was confirmed that the cloned *M. bovis* haemolysin gene encoded a structural subunit that was most probably a member of the RTX family of haemolytic enzymes.

Further sequence analysis has established that, like other members of the family, the *M. bovis* RTX A subunit gene is flanked by DNA sequences capable of encoding the RTX B,C and D proteins.

Conservation of the RTX A subunit among *M. bovis*

To determine whether the gene for the RTX A subunit was present in *M. bovis* strains representing the known pilus serotypes, a southern hybridisation analysis was performed using the coding region of the RTX A subunit as a probe.

Genomic DNA extracted from the seven serotype strains of *M. bovis* (15) was digested with *Eco*RV and separated using agarose gel electrophoresis. The DNA was transferred to a Hybond N+ filter (Amersham,

Little Chalfont, Buckinghamshire, U.K.) using a previously described method (9). The probe used was a PCR amplified product that contained all of the coding region from the A subunit of the RTX haemolysin of *M. bovis*. This fragment was labelled with α^{32}P-dATP using the Megaprime labelling system (Amersham, Little Chalfont, Buckinghamshire, U.K.) according to the manufacturers instructions. High stringency conditions were used (hybridisation temperature 68°C; 2 washes at room temperature in 2 × SSC / 0.1% SDS; 1 wash at 68°C in 0.1 × SSC / 0.1% SDS) and the resulting filters were exposed to autoradiographic film (Kodak, Rochester, New York, USA) for 5 to 24 hours before developing.

Results showed that the gene encoding the RTX A haemolysin subunit was conserved in all seven representative strains of *M. bovis* examined. Interestingly, each of these strains is known to display the haemolytic phenotype on horse blood agar. In contrast, the non-haemolytic *M. bovis* strain Gordon 26L3 did not hybridise to the RTX A gene probe possibly suggesting that *M. bovis* contains only a single structural gene responsible for the haemolytic phenotype detected on horse blood agar.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

References

1. Moore L. J., and A. W. D. Lepper. (1991). Vet Microbiol. 29:75-83.
2. Lehr, C. H., G. Jayappa and R. A. Goodnow. (1985). Cornell Vet. 75:484-492.
3. Pugh, G. W., D. E. Hughes and G. D. Booth. (1977). Am J Vet Res. 38:1519-1522.
4. Frank, S. K., and J. D. Gerber. (1981). J Clin Microbiol. 13(2):269-271.
5. Billson, F.M., J. L. Hodgson, A. W. Lepper, W. P. Michalski, C. L. Schwartzkoff, P. R. Lehrbach, and J. M. Tennent. (1994). FEMS Microbiol Lett. 124(1):69-73.
6. Elleman, T. C., P. A. Hoyne, and A. W. D. Lepper. (1990). Infect Immun. 58(6):1678-1684.
7. Woodcock, D. M., P. J. Crowther, J. Doherty, S. Jefferson, E. DeCruz, M. Noyer-Weidner, S. S. Smith, M. Z. Michael, and M. W. Graham. (1989). Nucleic Acids Res. 17(9):3469-3478.
8. Raleigh, E. A., K. Lech, and R. Brent. (1989). In Current Protocols in Molecular Biology eds. Ausubel, F. M. et al., Publishing Associates and Wiley Interscience; New York. Unit 1.4.
9. Sambrook, J., E. F. Fritsch, and T. Maniatis. (1989). Molecular Cloning: A Laboratory Manual, 2nd edition. Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York.
10. Dillela, A. G., and S. L. C. Woo. (1985). Focus. 7(2):1-5.
11. Hohn, B., and J. Collins. (1980). Gene. 11(3-4):291-298.
12. Narberhaus. F., K. Giebeler, and H. Bahl. (1992). J Bacteriol. 174(10):3290-3299.
13. Altschul, S. F., T. L. Madden, A. A. Schaffer, Z. Jinghui, Z. Zhang, W. Miller, and D. J. Lipman. (1997). Nucleic Acids Res. 25:3389-3402.
14. Bourgeau, G., H. Lapointe, P. Peloquin, and D. Mayrand. (1992). Infection and Immunity. 60(8):3186-3192.
15. Ausubel, F. M. R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl. (1994). Current Protocols in Molecular Biology. Green Publishing Associates, Inc. and John Wiley and Sons Inc. New York.
16. Wertman, K. F., A. R. Wyman, and D. Botstein. (1986). Gene. 49(2):253-262.

17. Bolivar F., R. L. Rodriguez, P. J. Greene, M. C. Betlach, H. L. Heynecker, and H. W. Boyer. (1977). Gene. 2(2):95-113.

18. Stuer, W., K. E. Jaeger, and U. K. Winkler. (1986). J Bacteriol. 168:1070-1074.

19. Winkler, U. K., and M. Stuckman. (1979). J Bacteriol. 138:663-670.

20. Upton, C., and J. T. Buckley. (1995). Trends Biochem Sci. 20(5):178-9.

21. Laemmli, U. K. (1970). Nature. 227(259):680-685.

22. Towbin, H., T. Staehlin, and J. Gordon. (1979). Proc Natl Acad Sci USA. 76(9):4350-4354.

23. Fifis, T., C. Costopoulos, and J. A. Vaughn. (1996). Vet Microbiol. 49:219-233.

24. Strauch, K. L., and J. Beckwith. (1988). Proc Natl Acad Sci USA. 85(5):1576-1580.

25. Gilaldi, M., C. I. Champion, D. A. Haake, D. R. Blanco, J. F. Miller, J. N. Miller, and M. A. Lovett. (1993). J Bacteriol. 175:4129-4136.

26. Mackman, N., J-M. Nicaud, L. Gray, and I. B. Holland. (1985). Mol Genet. 201:282-288.

27. Atwell, J. L., J. M. Tennent, A. W. Lepper and T. C. Elleman. (1994). J Bacteriol 176(16):4875-82 .

28. Edman, P., and C. Begg. (1967). Eur. J. Biochem. 1:80-91.

29. Hewick, R. M., M. W. Hunkapillar, L. E. Hood, and W. J. Dreyer. 1981. J Biol Chem. 256: 7990-7997

Claims:

1. A polypeptide, the polypeptide having an amino acid sequence as set out in SEQ. ID. NO. 1 from amino acid 37 to 1114, or a sequence having at least 50% identity thereto, or a functional fragment thereof.

2. A polypeptide as claimed in claim 1, the polypeptide having a sequence of at least 70% identity with the sequence shown in SEQ. ID. NO:1 from amino acid 37 to 1114.

3. A polypeptide as claimed in claim 1, the polypeptide having a sequence of at least 80% identity with the sequence shown in SEQ. ID. NO:1 from amino acid 37 to 1114.

4. A polypeptide as claimed in claim 1, the polypeptide having a sequence of at least 90% identity with the sequence shown in SEQ. ID. NO:1 from amino acid 37 to 1114.

5. A polypeptide as claimed in any one of claims 1 to 4, the polypeptide having protease activity.

6. A nucleic acid molecule, the nucleic acid molecule comprising a sequence encoding a polypeptide as claimed in any one of claims 1 to 5.

7. A nucleic acid molecule comprising a sequence as set out in SEQ. ID. NO:2 or a sequence having at least 60% identity thereto, or a sequence which hybridises thereto under stringent conditions.

8. A nucleic acid molecule as claimed in claim 7, the nucleic acid molecule comprising a sequence having least 70% identity with the sequence shown in SEQ. ID. NO:2.

9. A nucleic acid molecule as claimed in claim 7, the nucleic acid molecule comprising a sequence having least 80% identity with the sequence shown in SEQ. ID. NO:2.

10. A nucleic acid molecule as claimed in claim 7, the nucleic acid molecule comprising a sequence having least 90% identity with the sequence shown in SEQ. ID. NO:2.

11. A composition for use in raising an immune response in an animal, the composition comprising the polypeptide as claimed in any one of claims 1 to 5 or a nucleic acid sequence as claimed in claim 6 and optionally a carrier and/or adjuvant.

12. A polypeptide, the polypeptide having an amino acid sequence as set out in SEQ. ID. NO:3 from amino acid 26 to 616, or a sequence having at least 50% identity thereto, or a functional fragment thereof.

13. A polypeptide as claimed in claim 12, the polypeptide having a sequence of at least 70% identity with the sequence shown in SEQ. ID. NO:3 from amino acid 26 to 616.

14. A polypeptide as claimed in claim 12, the polypeptide having a sequence of at least 80% identity with the sequence shown in SEQ. ID. NO:3 from amino acid 26 to 616.

15. A polypeptide as claimed in claim 12, the polypeptide having a sequence of at least 90% identity with the sequence shown in SEQ. ID. NO:3 from amino acid 26 to 616.

16. A polypeptide as claimed in any one of claims 12 to 15, the polypeptide having lipase activity.

17. A nucleic acid molecule, the nucleic acid molecule comprising a sequence encoding a polypeptide of any one of claims 12 to 16.

18. A nucleic acid molecule comprising a sequence as set out in SEQ. ID. NO:4 or a sequence having at least 60% identity thereto, or a sequence which hybridises thereto under stringent conditions.

19. A nucleic acid molecule as claimed in claim 18, the nucleic acid molecule comprising a sequence having at least 70% identity with the sequence shown in SEQ. ID. NO:4.

20. A nucleic acid molecule as claimed in claim 18, the nucleic acid molecule comprising a sequence having at least 80% identity with the sequence shown in SEQ. ID. NO:4.

21. A nucleic acid molecule as claimed in claim 18, the nucleic acid molecule comprising a sequence having at least 90% identity with the sequence shown in SEQ. ID. NO:4.

22. A composition for use in raising an immune response in an animal, the composition comprising a polypeptide as claimed in any one of claims 12 to 16 or a nucleic acid sequence as claimed in claim 17 and optionally a carrier and/or adjuvant.

23. A polypeptide, the polypeptide having an amino acid sequence as set out in SEQ. ID. NO:5, or a sequence having at least 60% identity thereto, or a functional fragment thereof.

24. A polypeptide as claimed in claim 23, the polypeptide having a sequence of at least 70% identity with the sequence shown in SEQ. ID. NO:5.

25. A polypeptide as claimed in claim 23, the polypeptide having a sequence of at least 80% identity with the sequence shown in SEQ. ID. NO:5.

26. A polypeptide as claimed in claim 23, the polypeptide having a sequence of at least 90% identity with the sequence shown in SEQ. ID. NO:5.

27. A polypeptide as claimed in any one of claims 23 to 26, the polypeptide having haemolysin activity.

28. A nucleic acid molecule, the nucleic acid molecule comprising a sequence encoding a polypeptide of any one of claims 23 to 27.

29. A nucleic acid molecule comprising a sequence as set out in SEQ. ID. NO:6 or a sequence having at least 60% identity thereto, or a sequence which hybridises thereto under stringent conditions.

30. A nucleic acid molecule as claimed in claim 29, the nucleic acid molecule comprising a sequence having at least 70 % identity with the sequence shown in SEQ. ID. NO:6.

31. A nucleic acid molecule as claimed in claim 29, the nucleic acid molecule comprising a sequence having at least 80 % identity with the sequence shown in SEQ. ID. NO:6.

32. A nucleic acid molecule as claimed in claim 29, the nucleic acid molecule comprising a sequence having at least 90 % identity with the sequence shown in SEQ. ID. NO:6.

33. A composition for use in raising an immune response in an animal, the composition comprising a polypeptide of any one of claims 23 to 27 or a nucleic acid sequence of claim 28 and optionally a carrier and/or adjuvant.

34. A composition for use in raising an immune response in an animal directed against *Moraxella*, the composition comprising at least one polypeptide selected from the group consisting of a polypeptide as claimed in any one of claims 1 to 5, a polypeptide as claimed in any one of claims 12 to 16, and a polypeptide as claimed in any one of claims 23 to 27, and optionally including an adjuvant or carrier.

35. A composition as claimed in claim 34, the composition comprising a polypeptide as claimed in any one of claims 23 to 27 and either one of, or preferably both of, a polypeptide as claimed in any one of claims 1 to 5 and a polypeptide as claimed in any one of claims 12 to 16.

36. A composition as claimed in claim 34 or claim 35 wherein the *Moraxella* is *M. bovis or M. catarrhalis*.

37. A composition as claimed in claim 34 or claim 35 wherein the *Moraxella* is *M. bovis*.

38. An antibody raised against a polypeptide selected from the group consisting of a polypeptide as claimed in any one of claims 1 to 5, a polypeptide as claimed in any one of claims 12 to 16, and a polypeptide as claimed in any one of claims 23 to 27.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
8 March 2001 (08.03.2001)

PCT

(10) International Publication Number
WO 01/16172 A1

(51) International Patent Classification[7]: C07K 14/22, 16/12, A61K 38/48, 39/095, C12N 15/31

(21) International Application Number: PCT/AU00/01048

(22) International Filing Date: 31 August 2000 (31.08.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PQ 2571 31 August 1999 (31.08.1999) AU

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/16172 A1

(54) Title: VACCINE ANTIGENS OF MORAXELLA

(57) Abstract: The present invention relates to antigens of *Moraxella*, in particular, *Moraxella bovis*, nucleic acid sequences encoding these antigens and formulations for use in raising an immune response against *Moraxella*.

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1     ttctcatgtt tgacagctta tcatcgataa gctttaatgc ggtagtttat cacagttaaa ttgctaacgc agtcaggcac cgtgtatgaa
91    atctaacaat gcgctcatcg tcatcctcgg caccgtcacc ctggatgctg taggcatagg cttggttatg ccggtactgc cgggcctctt
181   gcgggatatc gtccattccg acagcatcgc cagtcactat ggcgtgctgc tagcgctata tgcgttgatg caatttctat gcgcacccgt
271   tctcggagca ctgtccgacc gctttggccg ccgcccagtc ctgctcgctt cgctacttgg agccactatc gactacgcga tcatggcgac
361   cacaccegtc ctgtggatca ataatttaatg aacatatata ctctatttaa tatttcttat ttattcgtaa tattgccata aaaataatac
451   attatttcta tattaactaa actgttaata tttgtaaata ataaacattt gtttatctaa aaaaataaat aatataaatc aagcaattac
541   aatcttattt ttgaaaatac aataatactg caattgctta atctagacat taagtttatt tttgattaaa attgccaaaa cttgtgtaaa
631   taagtttcac cgaattgata ctttaagggt atcaatattg caacatggta aatgarrgct atgttgttgg gcattgcara aatrgtctat
721   aataacttgt tatggatgat tgatggcaat gataaactta gtgacaatga taaacgcaaa gaggtgtaat atgtcattac aaactcaacc
1                                                                                   M  S  L  Q  T  Q
811   tgccaagaga gggttctatg ttaagccttt aagtatggct tgcatgctgg taattagtgc tagtagtacg gtaagttatg ccaactcagc
7     P  A  K  R  G  F  Y  V  K  P  L  S  M  A  C  M  L  V  I  S  A  S  S  T  V  S  Y  A  N  S
901   tccaatgatt gttgattcac agtacaatag ttctaaatac tctttctacg attactattt agatttcctt aaacgtttta gaccaactcc
37    A  P  M  I  V  D  S  Q  Y  N  S  S  K  Y  S  F  Y  D  Y  Y  L  D  F  L  K  R  F  R  P  T
991   aactccagtg ccaagccctg tgagaccggc tcctgaactc gttcgtccga ccccagcccc gattccggct ccaacgcctg tgccaacacc
67    P  T  P  V  P  S  P  V  R  P  A  P  E  L  V  R  P  T  P  A  P  I  P  A  P  T  P  V  P  T
1081  ggcaccaatt agtggcggta tatcaggtag ctatattgct ccagtatcgc catcagaggt gagacagcct gattacacaa gacgcgttca
97    P  A  P  I  S  G  G  I  S  G  S  Y  I  A  P  V  S  P  S  E  V  R  Q  P  D  Y  T  R  R  V
1171  agccaatcta aaacgcaacc aacctgcacc aagtgctggc acacgtacag gttatagtgt catggatacg tcaaataatt ctaatttgac
127   Q  A  N  L  K  R  N  Q  P  A  P  S  A  G  T  R  T  G  Y  S  V  M  D  T  S  N  N  S  N  L
1261  atctaaattt tatggcacaa ccgaagatgg ttatgccgag cgtcttgaca acctaaagaa caccattgat acacgtcaag ccaaagtagg
157   T  S  K  F  Y  G  T  T  E  D  G  Y  A  E  R  L  D  N  L  K  N  T  I  D  T  R  Q  A  K  V
1351  tgtgattgat acaggcatta accgcttcaa ccgagacttg gttggtgcaa atgtgcatga tacacagatt gagtgtgttt ctgctggacg
187   G  V  I  D  T  G  I  N  R  F  N  R  D  L  V  G  A  N  V  H  D  T  Q  I  E  C  V  S  A  G
1441  ttccacctgc tatacgccag aaaatgattc aggcattgtt gaaatcccaa caacctctgc tagtggtagt catggcaacc aaatggcggc
217   R  S  T  C  Y  T  P  E  N  D  S  G  I  V  E  I  P  T  T  S  A  S  G  S  H  G  N  Q  M  A
1531  tgtcatcgct ggtaacaacg gcatgaccaa cgccaaaatt tacggcagtg acagtattga tcgacgttca aatggtggca accatttctt
247   A  V  I  A  G  N  N  G  M  T  N  A  K  I  Y  G  S  D  S  I  D  R  R  S  N  G  G  N  H  F
1621  gatgatgcgt aagctgaacc aagaccatgg tgtcaagatt tttaacaact cttgggggttc taacaacact gaccaatggt actacgatgc
277   L  M  M  R  K  L  N  Q  D  H  G  V  K  I  F  N  N  S  W  G  S  N  N  T  D  Q  W  Y  Y  D
1711  tcagcgccta aattacaatc ctactacagg acagattaat ccaaatcctt acagaaccag tattaccaat gctgaagtga ctttgcctgt
307   A  Q  R  L  N  Y  N  P  T  T  G  Q  I  N  P  N  P  Y  R  T  S  I  T  N  A  E  V  T  L  P
1801  catcatgat cttattatga atcgtgactc gcttatcatt aaagcaacag gtaacgaagg cttgaacgat gctcatgatg aaaacctagc
337   V  I  H  D  L  I  M  N  R  D  S  L  I  I  K  A  T  G  N  E  G  L  N  D  A  H  D  E  N  L
1891  accgctcatg aacagcaact tcaaaaaagg tttcattact gtctcctcgc ctagagaaga ttttggtaaa gcgaatcatt gtggtcgaac
367   A  P  L  M  N  S  N  F  K  K  G  F  I  T  V  S  S  P  R  E  D  F  G  K  A  N  H  C  G  R
1981  tgccgaatgg tgtgtatccg caacatcatc tacccaaaat tacgccaacg atggcagact gagtagctat aagggtacat cacctgcaac
397   T  A  E  W  C  V  S  A  T  S  S  T  Q  N  Y  A  N  D  G  R  L  S  S  Y  K  G  T  S  P  A
2071  cgctcgtgtg tccggcacgg cagtgctcgt gcaatctgct tatccttgga tgaaaaatga aaatatctct caaaccattt tgggtactgc
427   T  A  R  V  S  G  T  A  V  L  V  Q  S  A  Y  P  W  M  K  N  E  N  I  S  Q  T  I  L  G  T
2161  caaggatttc tcagagatta ctgccaattc acctaatggc taccaaggac taagaaaggt tagtagattg ccatctggtt attacggctc
457   A  K  D  F  S  E  I  T  A  N  S  P  N  G  Y  Q  G  L  R  K  V  S  R  L  P  S  G  Y  Y  G
2251  ttattacact gacaatcagg gtaatttcta tgttcctggc aatgtcaatt gggaaaaccg tcgaattgtc gctaatcata acggcaagaa
487   S  Y  Y  T  D  N  Q  G  N  F  Y  V  P  G  N  V  N  W  E  N  R  R  I  V  A  N  H  N  G  K
2341  cattacatgg gaagatggtt ggggtttgtt agatccagaa gcggccgcta aaggttatgg tggtttctat tgggataatg tggaattaga
517   N  I  T  W  E  D  G  W  G  L  L  D  P  E  A  A  A  K  G  Y  G  G  F  Y  W  D  N  V  E  L
2431  cactaaaggc acgcctttat ctgtattcta caatgaccta aaaggtgata aaggctttac caaaaaaggt gaaggtaaac ttgtctttac
547   D  T  K  G  T  P  L  S  V  F  Y  N  D  L  K  G  D  K  G  F  T  K  K  G  E  G  K  L  V  F
2521  tggtaataat agctataaag gcgactctgt catcgagggt ggttcactag aagtaaatgg taacaacggt ggttcaacca tggttgttaa
577   T  G  N  N  S  Y  K  G  D  S  V  I  E  G  G  S  L  E  V  N  G  N  N  G  G  S  T  M  V  V
2611  aggtggtgaa ctaacaggtt atggtaatgt agctaatgtt cgtcaaacag gtggttgggt taacaacgaa ggtaacctaa acatcagagg
607   K  G  G  E  L  T  G  Y  G  N  V  A  N  V  R  Q  T  G  G  W  V  N  N  E  G  N  L  N  I  R
```

Figure 1

```
2701  tgactacaac atcaacactc aacgtggcgt ggatgctggt ctaaaagctc aatttggcaa catgcttacc gtggacggta aggccaaact
637   G  D  Y  N   I  N  T   Q  R  G   V  D  A  G   L  K  A   Q  F  G   N  M  L  T   V  D  G   K  A  K

2791  aggtggtaca ctaaatctaa ctggtgagac caaagatggt atcatcagca aatcaggtag ccgtagcact gtacttcgtg ctaagcgtgg
667   L  G  G  T   L  N  L   T  G  E   T  K  D  G   I  I  S   K  S  G   S  R  S  T   V  L  R   A  K  R

2881  tcttgaaggt caatttgaca attatcgttc aagcaaccca ttatttgaag taacaaatgt tgaatatacg ccagaagtag acagaaatgg
697   G  L  E  G   Q  F  D   N  Y  R   S  S  N  P   L  F  E   V  T  N   V  E  Y  T   P  E  V   D  R  N

2971  cagagtggta ggtggttcac gcacgaacaa tgacgtgcaa gtaactgcca aacgtctaag tgcaggcaat gttgtttatg gcatcagcat
727   G  R  V  V   G  G  S   R  T  N   N  D  V  Q   V  T  A   K  R  L   S  A  G  N   V  V  Y   G  I  S

3061  gaatgacagt ggtagccgtg ttgcacaaaa cctagacaaa gtacttaatg atttagataa aaaacaagaa acacaaggtt cactgaccag
757   M  N  D  S   G  S  R   V  A  Q   N  L  D  K   V  L  N   D  L  D   K  K  Q  E   T  Q  G   S  L  T

3151  tgatgagaag caatttgcta accgtgtatt cactggtttt gaaaacatga attctggtgc agaatctaaa ctttctacag taagcaccaa
787   S  D  E  K   Q  F  A   N  R  V   F  T  G  F   E  N  M   N  S  G   A  E  S  K   L  S  T   V  S  T

3241  ccgtgagcta tacaagcttg acccaacttt ctatgctgac agtgcattaa acgcagtaga agacagtgct aaccatgcaa ccgaatttgg
817   N  R  E  L   Y  K  L   D  P  T   F  Y  A  D   S  A  L   N  A  V   E  D  S  A   N  H  A   T  E  F

3331  taagcgtgtt agcgccccaa gaggtgtttg gggtaatatc agtcaccatg attatgatgt agaactagag catgctacaa gtgcacgtaa
847   G  K  R  V   S  A  P   R  G  V   W  G  N  I   S  H  H   D  Y  D   V  E  L  E   H  A  T   S  A  R

3421  aggcaacaac attagtgttg gtgcaagcac tcaaactgca gccgacatta gtgttggtgc acaacttgat gtaagtaaac ttgacttgga
877   K  G  N  N   I  S  V   G  A  S   T  Q  T  A   A  D  I   S  V  G   A  Q  L  D   V  S  K   L  D  L

3511  agaatctgtt tatggtattg gcaacaaaac caaaactgac agcattggct tgactgttgg tgcttctaag aagttgggtg atgcctatct
907   E  E  S  V   Y  G  I   G  N  K   T  K  T  D   S  I  G   L  T  V   G  A  S  K   K  L  G   D  A  Y

3601  atcaggttgg gtaaaaggtg ccaaagttga tacagaagcg aaccgtggtg aaaactctaa caaagttgag tacaatggta agctatatgg
937   L  S  G  W   V  K  G   A  K  V   D  T  E  A   N  R  G   E  N  S   N  K  V  E   Y  N  G   K  L  Y

3691  tgctggtatc caagcgggta caaacattga tactgcatcg ggcgtgagtg tacaacctta tgcctttgtt aaccatcagc agtacaaaaa
967   G  A  G  I   Q  A  G   T  N  I   D  T  A  S   G  V  S   V  Q  P   Y  A  F  V   N  H  Q   Q  Y  K

3781  cgatggtagc ttcaatgacg gtcttaacgt tgttgacgac atcgaagcaa aacaaactca ggtaggtgtg ggtgctgata tggtgttcca
997   N  D  G  S   F  N  D   G  L  N   V  V  D  D   I  E  A   K  Q  T   Q  V  G  V   G  A  D   M  V  F

3871  agcaacacct gctctacagc ttactggtgg tgtgcaagtt gctcacgctg ttagccgtga caccaaccta gacactcgct atgttggtac
1027  Q  A  T  P   A  L  Q   L  T  G   G  V  Q  V   A  H  A   V  S  R   D  T  N  L   D  T  R   Y  V  G

3961  agcgacagat gtacagtatg gcacttggga tactgacaaa accaaatggt cagccaaggt tggtgctaac tataatgtga caccaaacag
1057  T  A  T  D   V  Q  Y   G  T  W   D  T  D  K   T  K  W   S  A  K   V  G  A  N   Y  N  V   T  P  N

4051  ccaagtgggt ctaaactaca gctacacagg tagtggcgat tcagatgctt cccaagtggg tgtgagcttc accagcaagt tctaattcat
1087  S  Q  V  G   L  N  Y   S  Y  T   G  S  G  D   S  D  A   S  Q  V   G  V  S  F   T  S  K   F  -

4141  taataaggca acaaaaaaca gcacaatttc ggttgtgctg ttttttgtga tgccgagcgt aaaattttcc caaaaaaagc gtgataatta

4231  ccacgctttt ttattgcata ttgcaaaata gtattgcatt tatgggttgt taagcaaccc gtccaaatac cccctaaaca actccacccc

4321  aatcggtgct aacttgtttt gccacaggct cgtcaatgtg tcggcatcat caaccattac cgac
```

Figure 1 (continued)

```
   1  tgggcagata acccatcaaa gacccaaagc aacccataaa tcaaaaaaac acttgtaatt tgtgtaatat cttgttacac tttacaagtg

  91  tttttacttt gaaagcaact cagtgagtaa taatgaaaaa atccgccttt gccaaatact cagcacttgc cctaatggtt gggatgtgcc
   1                                  M  K  K  S  A  F  A  K  Y  S  A  L  A  L  M  V  G  M  C

 181  tgcacaccgc ttacgccaag gagtttagcc aagtcatcat ttttggggac agcttgtccg atacaggtcg cctaaaagat atggtcgccc
  20  L  H  T  A  Y  A  K  E  F  S  Q  V  I  I  F  G  D  S  L  S  D  T  G  R  L  K  D  M  V  A

 271  gaaaagatgg cacccttggc aacaccttac agccatcttt taccaccaac cccgaccctg tatggtcaag cttattttgcc caaagttatg
  50  R  K  D  G  T  L  G  N  T  L  Q  P  S  F  T  T  N  P  D  P  V  W  S  S  L  F  A  Q  S  Y

 361  gcaaaaccgc cagtgccaac acgccctaca atcccactgg cactaactat gccgtgggcg gagctcgctc tggctcggag gtcaattgga
  80  G  K  T  A  S  A  N  T  P  Y  N  P  T  G  T  N  Y  A  V  G  G  A  R  S  G  S  E  V  N  W

 451  atggttttgt gaatgtaccc tccaccaaaa cgcaaatcac cgaccatttg accgccacag gtggcaaagc cgaccctaat accctgtatg
 110  N  G  F  V  N  V  P  S  T  K  T  Q  I  T  D  H  L  T  A  T  G  G  K  A  D  P  N  T  L  Y

 541  ccatttggat tggctctaat gacttaattt cagcttctca agccaccaca acagccgaag cccaaaacgc cattaaaggt gcggtaactc
 140  A  I  W  I  G  S  N  D  L  I  S  A  S  Q  A  T  T  T  A  E  A  Q  N  A  I  K  G  A  V  T

 631  gcaccgtgat agacatcgaa acactcaatc aagcaggggc gacaaccatt ttggtgccaa atgtgcctga tttgagcctc acgccccgag
 170  R  T  V  I  D  I  E  T  L  N  Q  A  G  A  T  T  I  L  V  P  N  V  P  D  L  S  L  T  P  R

 721  ccatctatgg cgaaagcctc atggcaggcg tgcaagacaa agccaaactc gcctcaagtc tgtataatag cggtctgttt gaagcattaa
 200  A  I  Y  G  E  S  L  M  A  G  V  Q  D  K  A  K  L  A  S  S  L  Y  N  S  G  L  F  E  A  L

 811  atcaatccac cgccaacatc atcccctgcca acacctttgc cctactccaa gaagcgacca caaataaaga agcctttggt tttaaaaaca
 230  N  Q  S  T  A  N  I  I  P  A  N  T  F  A  L  L  Q  E  A  T  T  N  K  E  A  F  G  F  K  N

 901  cgcaaggcgt ggcgtgtcaa atgcccgctc gtaccacagg ggcggatgat gtggcttcta cttccttggc atgtaccaaa gccaatctta
 260  T  Q  G  V  A  C  Q  M  P  A  R  T  T  G  A  D  D  V  A  S  T  S  L  A  C  T  K  A  N  L

 991  tagaaaacgg ggcaaatgac acctacgcct ttgccgatga cattcaccca tcgggacgca cgcaccgcat tttggcacag tattaccgtt
 290  I  E  N  G  A  N  D  T  Y  A  F  A  D  D  I  H  P  S  G  R  T  H  R  I  L  A  Q  Y  Y  R

1081  ctatcatgga cgcccctact cacatgggta aactctcagg cgagcttgtc aaaacaggtt cagcccacga ccgtcatgtt taccgtcagc
 320  S  I  M  D  A  P  T  H  M  G  K  L  S  G  E  L  V  K  T  G  S  A  H  D  R  H  V  Y  R  Q

1171  ttgacaggct tagtggctca cagcacagca tttgggcaaa cgtccatgcc agcgaccgta ccgaccccac cacccaaatc ggcttggacg
 350  L  D  R  L  S  G  S  Q  H  S  I  W  A  N  V  H  A  S  D  R  T  D  P  T  T  Q  I  G  L  D

1261  tggcaggttc atcaagccat acaggggcgt atctgagcca ccaaaaccaa gattatgtgc tggatgacac cctatcatca gatgtcaaaa
 380  V  A  G  S  S  S  H  T  G  A  Y  L  S  H  Q  N  Q  D  Y  V  L  D  D  T  L  S  S  D  V  K

1351  ccattggcat ggggctgtat catcgccatg acatcggcaa tgtccgtcta aaaggcgtgg caggtatcga ccgacttagc gtggatacgc
 410  T  I  G  M  G  L  Y  H  R  H  D  I  G  N  V  R  L  K  G  V  A  G  I  D  R  L  S  V  D  T

1441  accgccatat cgactgggag ggggcaagcc gttcgcacac ggcagacacc accgccagac gttttcatgc agggctacaa gccagctatg
 440  H  R  H  I  D  W  E  G  A  S  R  S  H  T  A  D  T  T  A  R  R  F  H  A  G  L  Q  A  S  Y

1531  gcatagacat gggcaaagcc accgtgcgtc cgcttatcgg cgtacatgcc caaaaagtca aagtgcgtga tttggtagag aatgagccta
 470  G  I  D  M  G  K  A  T  V  R  P  L  I  G  V  H  A  Q  K  V  K  V  R  D  L  V  E  N  E  P

1621  ccctatccac cgccatgcgt tttggcgagc aagaacaaaa gtcctacaa ggcgagattg gcgtcgatgt ggcttatccg attagccctg
 500  T  L  S  T  A  M  R  F  G  E  Q  E  Q  K  S  L  Q  G  E  I  G  V  D  V  A  Y  P  I  S  P

1711  ctttgactct gacgggcggt atcgctcacg ctcatgagtt taacgatgat gaacgcacca ttaatgccac tttaacctcc attcgtgaat
 530  A  L  T  L  T  G  G  I  A  H  A  H  E  F  N  D  D  E  R  T  I  N  A  T  L  T  S  I  R  E

1801  acacgaaggg ctttaataca agcgttagca ccgacaaatc tcacgccacc accgctcatc tgggcgtaca agggcaactt ggcaaggcaa
 560  Y  T  K  G  F  N  T  S  V  S  T  D  K  S  H  A  T  T  A  H  L  G  V  Q  G  Q  L  G  K  A

1891  atattcatgc aggcgttcac gccacccacc aagacagcga tacagacgtg ggtggttcgc ttggggttcg cttgatgttt tgattggctt
 590  N  I  H  A  G  V  H  A  T  H  Q  D  S  D  T  D  V  G  G  S  L  G  V  R  L  M  F  -

1981  ttaaagataa aaagtggtat catgccactt tttattttgc caaaaatcta tgtttgagta catcaaagcc tttcacatca tcgccatgcg

2071  atgataagct gtcaaacatg ag
```

Figure 2

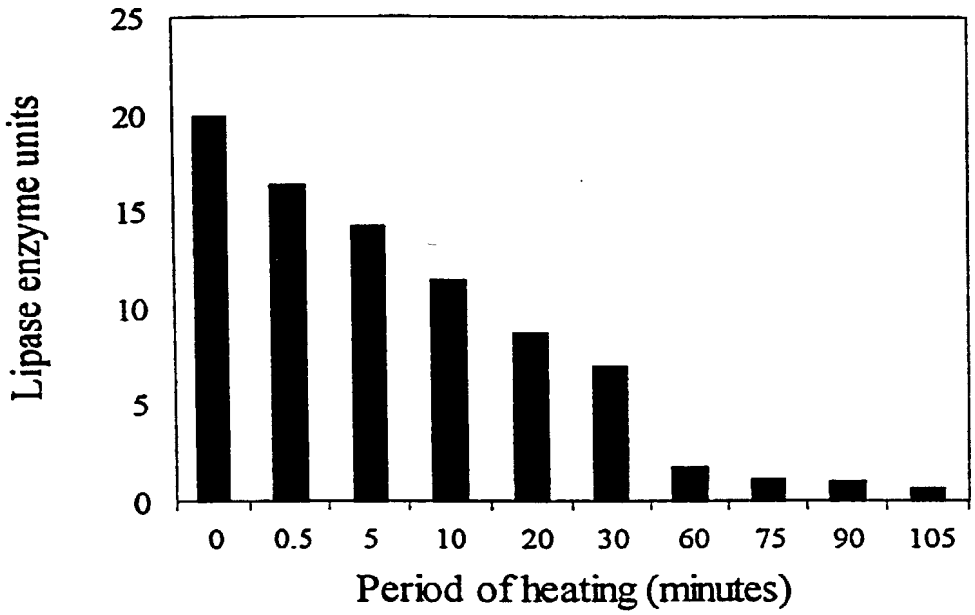
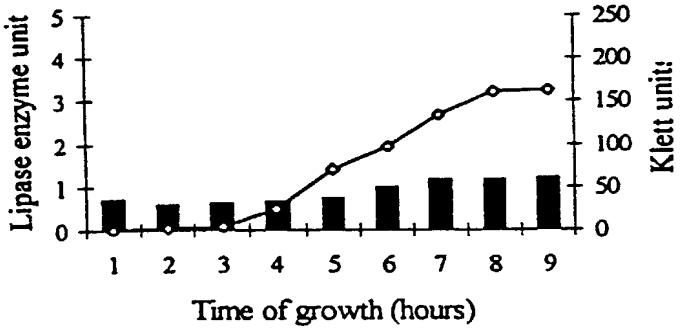

25
20
15
10
5
0
Lipase enzyme units
0
0.5
5
10
20
30
60
75
90
105
Period of heating (minutes)

Figure 3

(i) Dalton 2d / *M. bovis*



(ii) pMB1 / MC1061

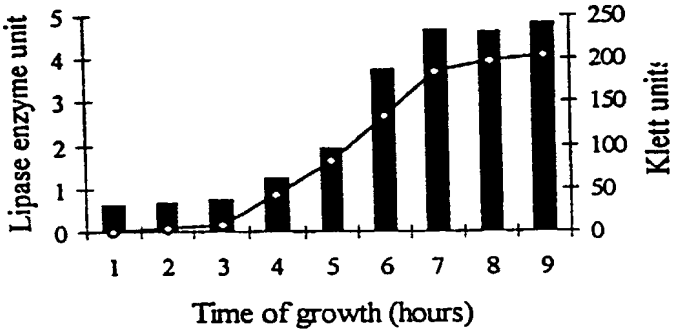


Figure 4

```
1    atgagaacgt tattttcaga tgaattgttt agagcgattc gtgtagatgg aaatttcatcg catggtaaga tatctgaatt ttatggaaag
       M  R  T  L  F  S  D  E  L  F  R  A  I  R  V  D  G  N  S  S  H  G  K  I  S  E  F  Y  G  K

91   tctgttgatt caaaattagc ctcaagaata tttgcacaat atcacgaaga tttgacgagc aaattgtcaa ctcagaataa ttttattata
       S  V  D  S  K  L  A  S  R  I  F  A  Q  Y  H  E  D  L  T  S  K  L  S  T  Q  N  N  F  I  I

181  tctaaagata attaatacaa ccttttctaa cacaacgagg agaagcatat tatgtccaat ataaatgtaa ttaaatctaa tattcaagca
1      S  K  D  N  -                                                  M  S  N  I  N  V  I  K  S  N  I  Q  A

271  ggcttgaatt caacaaagtc tggattaaaa aatctttact tggctattcc caaagattat gatccgcaaa aaggtgggac tttaaatgat
14     G  L  N  S  T  K  S  G  L  K  N  L  Y  L  A  I  P  K  D  Y  D  P  Q  K  G  G  T  L  N  D

361  tttattaaag ctgctgatga attaggtatt gctcgtttag cagaagagcc taatcacact gaaacagcaa aaaaatctgt tgacacagta
44     F  I  K  A  A  D  E  L  G  I  A  R  L  A  E  E  P  N  H  T  E  T  A  K  K  S  V  D  T  V

451  aatcagtttc tctctctcac acaaactggt attgctattt ctgcaacaaa attagaaaag ttcttacaaa aacattctac caataagtta
74     N  Q  F  L  S  L  T  Q  T  G  I  A  I  S  A  T  K  L  E  K  F  L  Q  K  H  S  T  N  K  L

541  gccaaagggt tagacagtgt agaaaatatt gatcgtaaat taggtaaagc aagtaatgta ttatcaacat taagctcttt tttgggcact
104    A  K  G  L  D  S  V  E  N  I  D  R  K  L  G  K  A  S  N  V  L  S  T  L  S  S  F  L  G  T

631  gcattagcgg gtatagaact tgattcttta atcaaaaaag gtgatgctgc acctgatgct ttggctaaag ctagtattga cttgattaat
134    A  L  A  G  I  E  L  D  S  L  I  K  K  G  D  A  A  P  D  A  L  A  K  A  S  I  D  L  I  N

721  gagataattg gtaatctatc tcagagtact caaacgattg aagcattttc ttcacagtta gcaaagttag gttctactat atcgcaggct
164    E  I  I  G  N  L  S  Q  S  T  Q  T  I  E  A  F  S  S  Q  L  A  K  L  G  S  T  I  S  Q  A

811  aaaggcttct ctaatatagg aaacaagttg caaaacttaa atttttctaa aacaaatctt ggtttggaaa taattactgg tttgctatca
194    K  G  F  S  N  I  G  N  K  L  Q  N  L  N  F  S  K  T  N  L  G  L  E  I  I  T  G  L  L  S

901  ggcatttctg caggctttgc tttagcggat aaaaatgcat cgactggcaa aaaagttgct gcaggttttg aattaagcaa tcaagttatt
224    G  I  S  A  G  F  A  L  A  D  K  N  A  S  T  G  K  K  V  A  A  G  F  E  L  S  N  Q  V  I

991  ggtaatgtaa caaaagcaat ttcttcatat gttttagcac aacgtgttgc tgctggtcta tcaactactg gtgctgttgc tgctttaatt
254    G  N  V  T  K  A  I  S  S  Y  V  L  A  Q  R  V  A  A  G  L  S  T  T  G  A  V  A  A  L  I

1081 acttcatcga ttatgttggc aattagtcct ttggcattta tgaatgcagc agataaattc aatcatgcta atgctcttga tgagtttgca
284    T  S  S  I  M  L  A  I  S  P  L  A  F  M  N  A  A  D  K  F  N  H  A  N  A  L  D  E  F  A

1171 aaacaattcc gaaaatttgg ctatgatggg gatcatttat tggctgaata tcagcgtggt gtgggtacta ttgaagcttc attaactaca
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1261 attagtacgg cattaggtgc agtttctgct ggtgtttccg ctgctgctgt aggatctgct gttggtacac cgattgcact attagttgca
344    I  S  T  A  L  G  A  V  S  A  G  V  S  A  A  A  V  G  S  A  V  G  T  P  I  A  L  L  V  A

1351 ggtgttacag gattgatctc tggaatttta gaagcgtcta aacaggcaat gtttgaaagt gttgctaacc gtttacaagg taaaatttta
374    G  V  T  G  L  I  S  G  I  L  E  A  S  K  Q  A  M  F  E  S  V  A  N  R  L  Q  G  K  I  L

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404    E  W  E  K  Q  N  G  G  Q  N  Y  F  D  K  G  Y  D  S  R  Y  A  A  Y  L  A  N  N  L  K  F

1531 ttgtctgagc taaataaaga gttggaagct gaacgtgtta ttgcaatcac ccaacaacgt tgggataata atattggtga gttagcaggt
434    L  S  E  L  N  K  E  L  E  A  E  R  V  I  A  I  T  Q  Q  R  W  D  N  N  I  G  E  L  A  G

1621 attaccaaat tgggtgaacg cattaagagc ggaaaagctt atgcagatgc ttttgaagat ggcaagaaag ttgaagctgg ttccaatatt
464    I  T  K  L  G  E  R  I  K  S  G  K  A  Y  A  D  A  F  E  D  G  K  K  V  E  A  G  S  N  I

1711 actttggatg ctaaaactgg tatcatagac attagtaatt caaatgggaa aaaaacgcaa gcgttgcatt tcacttcgcc tttgttaaca
494    T  L  D  A  K  T  G  I  I  D  I  S  N  S  N  G  K  K  T  Q  A  L  H  F  T  S  P  L  L  T

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524    A  G  T  E  S  R  E  R  L  T  N  G  K  Y  S  Y  I  N  K  L  K  F  G  R  V  K  N  W  Q  V

1891 acagatggag aggctagttc taaattagat ttctctaaag ttattcagcg tgtagccgag acagaaggca cagacgagat tggtctaata
554    T  D  G  E  A  S  S  K  L  D  F  S  K  V  I  Q  R  V  A  E  T  E  G  T  D  E  I  G  L  I

1981 gtaaatgcaa aagctggcaa tgacgatatc tttgttggtc aaggtaaaat gaatattgat ggtggagatg gacacgatcg tgtctttctat
584    V  N  A  K  A  G  N  D  D  I  F  V  G  Q  G  K  M  N  I  D  G  G  D  G  H  D  R  V  F  Y

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Figure 5

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 794    M  I  E   R  T  K   E  G  I  I   V  K  R   N  D  H   S  G  S  I   N  I  P   R  W  Y   I  T  S  N

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 824    L  Q  N   Y  Q  S   N  K  T  D   H  K  I   E  Q  L   I  G  K  D   G  S  Y   I  T  S   D  Q  I  D

2791  aaaattttgc aagataagaa agatggtaca gtaattacat ctcaagaatt gaaaaagctt gctgatgaga ataagagcca aaaatttatct
 854    K  I  L   Q  D  K   K  D  G  T   V  I  T   S  Q  E   L  K  K  L   A  D  E   N  K  S   Q  K  L  S

2881  gcttcggaca ttgcaagtag cttaaataag ctagttgggt caatggcact atttggtaca gcaaatagtg tgagttctaa cgccttacag
 884    A  S  D   I  A  S   S  L  N  K   L  V  G   S  M  A   L  F  G  T   A  N  S   V  S  S   N  A  L  Q

2971  ccaattacac aaccaactca aggaattttg gctccaagtg tttagtgatt taatttacta gacaatatca ccacccatat cattggttat
 914    P  I  T   Q  P  T   Q  G  I  L   A  P  S   V  -

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                            M  G  G   D  T  S  L   I  R  L   N  L  Q   T  L  N  S   N  L  V   M  I  D

3151  tgctcaacaa cctgctctat ctgctctggt tatccttgcc aaatactatg gtatttctgc aagtccagca gacattatgc atcgactggc
      Y  A  Q  Q   P  A  L   S  A  L   V  I  L  A   K  Y  Y   G  I  S   A  S  P  A   D  I  M   H  R  L

3241  aaaaaagttg
      A  K  K  L
```

Figure 5 (continued)

Attorney Docket No. **20-02**

JOINT INVENTORS' DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY

As the below named inventors, we hereby declare that:

Our residences, post office addresses and citizenship are as stated below our names.

We believe that we are the original, first and joint inventors of the subject matter which is claimed and for which a patent is sought on the invention entitled: Vaccine Antigens of Moraxella.

the specification of which:

____ is attached hereto;

__X__ was filed on February 28, 2002 as Application Serial No. 10/069,799.

We hereby authorize our legal representative to add reference to the Serial No. and/or filing date of the above-referenced application to this declaration.

We hereby state that we have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

We acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, §1.56.

Prior Foreign Application(s)

We hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application(s) for patent or inventor's certificate having a filing date before that of the application to which priority is claimed:

Country	Application No.	Date of Filing (day,month,year)	Date of Issue (day,month,year)	Priority Claimed 35 U.S.C.119
Australia	**PQ2571**	**31 August 1999**		Yes X No __

Prior Provisional Application(s)

We hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below:

Application Serial Number	Date of Filing (day,month,year)

Prior U.S. Application(s) and PCT International Application(s) Designating the United States

We hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s), or § 365(c) of any PCT International application(s) designating the United States listed below:

Application Serial Number	Date of Filing (day,month,year)	Status (Patented, Pending, Abandoned)
PCT/AU00/01048	**31 August 2000**	**Pending**

Insofar as the subject matter of each of the claims in this application is not disclosed in the prior United States, foreign or PCT International application(s) to which priority has been claimed above in the manner provided by the first paragraph of Title 35, United States Code, §112, we acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56 which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application.

We hereby appoint, both jointly and severally, as our attorneys and agents with full power of substitution and revocation, to prosecute this application and any corresponding application filed in the Patent Cooperation Treaty Receiving Office, and to transact all business in the Patent and Trademark Office connected herewith the following attorneys and agents, their registration numbers being listed after their names:

(10) Lorance L. Greenlee, Reg. No. 27,894; Ellen P. Winner, Reg. No. 28,547; Sally A. Sullivan, Reg. No. 32,064; Donna M. Ferber, Reg. No. 33,878; G. William Van Cleave, Reg. No. 40,213; Susan K. Doughty, Reg. No. 43,595; Heeja Yoo-Warren, Reg. No. 45,495, Tamala R. Jonas, Reg. No. 47,688, Mary Beth Vellequette, Reg. No. 47,903 and Jonathan A. Baker, Reg. No. 49,022 all of Greenlee, Winner and Sullivan, P.C., 5370 Manhattan Circle, Suite 201, Boulder, CO 80303.

We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

1-00

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AUSTRALIA

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2-00

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3-00

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SEQUENCE LISTING

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Commonwealth Scientific and Industrial Research Organisation

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Pro Asn Pro Tyr Arg Thr Ser Ile Thr Asn Ala Glu Val Thr Leu Pro
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Ala Arg Lys Asp Gly Thr Leu Gly Asn Thr Leu Gln Pro Ser Phe Thr
50 55 60

Thr Asn Pro Asp Pro Val Trp Ser Ser Leu Phe Ala Gln Ser Tyr Gly
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Lys Thr Ala Ser Ala Asn Thr Pro Tyr Asn Pro Thr Gly Thr Asn Tyr
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Ala Val Gly Gly Ala Arg Ser Gly Ser Glu Val Asn Trp Asn Gly Phe
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Val Asn Val Pro Ser Thr Lys Thr Gln Ile Thr Asp His Leu Thr Ala
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Thr Gly Gly Lys Ala Asp Pro Asn Thr Leu Tyr Ala Ile Trp Ile Gly
130 135 140

Ser Asn Asp Leu Ile Ser Ala Ser Gln Ala Thr Thr Thr Ala Glu Ala
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Gln Asn Ala Ile Lys Gly Ala Val Thr Arg Thr Val Ile Asp Ile Glu
165 170 175

Thr Leu Asn Glu Ala Gly Ala Thr Thr Ile Leu Val Pro Asn Val Pro
180 185 190

Asp Leu Ser Leu Thr Pro Arg Ala Ile Tyr Gly Glu Ser Leu Met Ala
195 200 205

Gly Val Gln Asp Lys Ala Lys Leu Ala Ser Ser Leu Tyr Asn Ser Gly
210 215 220

Leu Phe Glu Ala Leu Asn Gln Ser Thr Ala Asn Ile Ile Pro Ala Asn
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Thr Phe Ala Leu Leu Gln Glu Ala Thr Thr Asn Lys Glu Ala Phe Gly
245 250 255

Phe Lys Asn Thr Gln Gly Val Ala Cys Gln Met Pro Ala Arg Thr Thr
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Gly Ala Asp Asp Val Ala Ser Thr Ser Leu Ala Cys Thr Lys Ala Asn
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Leu Ile Glu Asn Gly Ala Asn Asp Thr Tyr Ala Phe Ala Asp Asp Ile
290 295 300

His Pro Ser Gly Arg Thr His Arg Ile Leu Ala Gln Tyr Tyr Arg Ser
305 310 315 320

Ile Met Asp Ala Pro Thr His Met Gly Lys Leu Ser Gly Glu Leu Val
325 330 335

Lys Thr Gly Ser Ala His Asp Arg His Val Tyr Arg Gln Leu Asp Arg
340 345 350

Leu Ser Gly Ser Gln His Ser Ile Trp Ala Asn Val His Ala Ser Asp
355 360 365

Arg Thr Asp Pro Thr Thr Gln Ile Gly Leu Asp Val Ala Gly Ser Ser
370 375 380

Ser His Thr Gly Ala Tyr Leu Ser His Gln Asn Gln Asp Tyr Val Leu
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Asp Asp Thr Leu Ser Ser Asp Val Lys Thr Ile Gly Met Gly Leu Tyr
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His Arg His Asp Ile Gly Asn Val Arg Leu Lys Gly Val Ala Gly Ile
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Ser Arg Ser His Thr Ala Asp Thr Thr Ala Arg Arg Phe His Ala Gly
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Leu Gln Ala Ser Tyr Gly Ile Asp Met Gly Lys Ala Thr Val Arg Pro
465                 470                 475                 480

Leu Ile Gly Val His Ala Gln Lys Val Lys Val Arg Asp Leu Val Glu
                485                 490                 495

Asn Glu Pro Thr Leu Ser Thr Ala Met Arg Phe Gly Glu Gln Glu Gln
            500                 505                 510

Lys Ser Leu Gln Gly Glu Ile Gly Val Asp Val Ala Tyr Pro Ile Ser
        515                 520                 525

Pro Ala Leu Thr Leu Thr Gly Gly Ile Ala His Ala His Glu Phe Asn
    530                 535                 540

Asp Asp Glu Arg Thr Ile Asn Ala Thr Leu Thr Ser Ile Arg Glu Tyr
545                 550                 555                 560

Thr Lys Gly Phe Asn Thr Ser Val Ser Thr Asp Lys Ser His Ala Thr
                565                 570                 575

Thr Ala His Leu Gly Val Gln Gly Gln Leu Gly Lys Ala Asn Ile His
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Tyr Asp Pro Gln Lys Gly Gly Thr Leu Asn Asp Phe Ile Lys Ala Ala
35 40 45

Asp Glu Leu Gly Ile Ala Arg Leu Ala Glu Glu Pro Asn His Thr Glu
50 55 60

Thr Ala Lys Lys Ser Val Asp Thr Val Asn Gln Phe Leu Ser Leu Thr
65 70 75 80

Gln Thr Gly Ile Ala Ile Ser Ala Thr Lys Leu Glu Lys Phe Leu Gln
85 90 95

Lys His Ser Thr Asn Lys Leu Ala Lys Gly Leu Asp Ser Val Glu Asn
100 105 110

Ile Asp Arg Lys Leu Gly Lys Ala Ser Asn Val Leu Ser Thr Leu Ser
115 120 125

Ser Phe Leu Gly Thr Ala Leu Ala Gly Ile Glu Leu Asp Ser Leu Ile
130 135 140

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Lys Lys Gly Asp Ala Ala Pro Asp Ala Leu Ala Lys Ala Ser Ile Asp
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Leu Ile Asn Glu Ile Ile Gly Asn Leu Ser Gln Ser Thr Gln Thr Ile
                165                 170                 175

Glu Ala Phe Ser Ser Gln Leu Ala Lys Leu Gly Ser Thr Ile Ser Gln
            180                 185                 190

Ala Lys Gly Phe Ser Asn Ile Gly Asn Lys Leu Gln Asn Leu Asn Phe
        195                 200                 205

Ser Lys Thr Asn Leu Gly Leu Glu Ile Ile Thr Gly Leu Leu Ser Gly
    210                 215                 220

Ile Ser Ala Gly Phe Ala Leu Ala Asp Lys Asn Ala Ser Thr Gly Lys
225                 230                 235                 240

Lys Val Ala Ala Gly Phe Glu Leu Ser Asn Gln Val Ile Gly Asn Val
                245                 250                 255

Thr Lys Ala Ile Ser Ser Tyr Val Leu Ala Gln Arg Val Ala Ala Gly
            260                 265                 270

Leu Ser Thr Thr Gly Ala Val Ala Ala Leu Ile Thr Ser Ser Ile Met
        275                 280                 285

Leu Ala Ile Ser Pro Leu Ala Phe Met Asn Ala Ala Asp Lys Phe Asn
    290                 295                 300

His Ala Asn Ala Leu Asp Glu Phe Ala Lys Gln Phe Arg Lys Phe Gly
305                 310                 315                 320

Tyr Asp Gly Asp His Leu Leu Ala Glu Tyr Gln Arg Gly Val Gly Thr
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Ile Glu Ala Ser Leu Thr Thr Ile Ser Thr Ala Leu Gly Ala Val Ser
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Ala Gly Val Ser Ala Ala Ala Val Gly Ser Ala Val Gly Thr Pro Ile
        355                 360                 365

Ala Leu Leu Val Ala Gly Val Thr Gly Leu Ile Ser Gly Ile Leu Glu
    370                 375                 380

Ala Ser Lys Gln Ala Met Phe Glu Ser Val Ala Asn Arg Leu Gln Gly
385                 390                 395                 400

Lys Ile Leu Glu Trp Glu Lys Gln Asn Gly Gly Gln Asn Tyr Phe Asp
                405                 410                 415

Lys Gly Tyr Asp Ser Arg Tyr Ala Ala Tyr Leu Ala Asn Asn Leu Lys
            420                 425                 430

Phe Leu Ser Glu Leu Asn Lys Glu Leu Glu Ala Glu Arg Val Ile Ala
        435                 440                 445
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Ile Thr Gln Gln Arg Trp Asp Asn Asn Ile Gly Glu Leu Ala Gly Ile
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Thr Lys Leu Gly Glu Arg Ile Lys Ser Gly Lys Ala Tyr Ala Asp Ala
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Phe Glu Asp Gly Lys Lys Val Glu Ala Gly Ser Asn Ile Thr Leu Asp
                485                 490                 495

Ala Lys Thr Gly Ile Ile Asp Ile Ser Asn Ser Asn Gly Lys Lys Thr
            500                 505                 510

Gln Ala Leu His Phe Thr Ser Pro Leu Leu Thr Ala Gly Thr Glu Ser
        515                 520                 525

Arg Glu Arg Leu Thr Asn Gly Lys Tyr Ser Tyr Ile Asn Lys Leu Lys
    530                 535                 540

Phe Gly Arg Val Lys Asn Trp Gln Val Thr Asp Gly Glu Ala Ser Ser
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Lys Leu Asp Phe Ser Lys Val Ile Gln Arg Val Ala Glu Thr Glu Gly
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Thr Asp Glu Ile Gly Leu Ile Val Asn Ala Lys Ala Gly Asn Asp Asp
            580                 585                 590

Ile Phe Val Gly Gln Gly Lys Met Asn Ile Asp Gly Gly Asp Gly His
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Asp Arg Val Phe Tyr Ser Lys Asp Gly Gly Phe Gly Asn Ile Thr Val
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Asp Gly Thr Ser Ala Thr Glu Ala Gly Ser Tyr Thr Val Asn Arg Lys
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Val Ala Arg Gly Asp Ile Tyr His Glu Val Val Lys Arg Gln Glu Thr
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Lys Val Gly Lys Arg Thr Glu Thr Ile Gln Tyr Arg Asp Tyr Glu Leu
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Arg Lys Val Gly Tyr Gly Tyr Gln Ser Thr Asp Asn Leu Lys Ser Val
        675                 680                 685

Glu Glu Val Ile Gly Ser Gln Phe Asn Asp Val Phe Lys Gly Ser Lys
    690                 695                 700

Phe Asn Asp Ile Phe His Ser Gly Glu Gly Asp Asp Leu Leu Asp Gly
705                 710                 715                 720

Gly Ala Gly Asp Asp Arg Leu Phe Gly Gly Lys Gly Asn Asp Arg Leu
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Ser Gly Asp Glu Gly Asp Asp Leu Leu Asp Gly Gly Ser Gly Asp Asp
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Val Leu Asn Gly Gly Ala Gly Asn Asp Val Tyr Ile Phe Arg Lys Gly
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Asp Gly Asn Asp Thr Leu Tyr Asp Gly Thr Gly Asn Asp Lys Leu Ala
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Phe Ala Asp Ala Asn Ile Ser Asp Ile Met Ile Glu Arg Thr Lys Glu
785                 790                 795                 800

Gly Ile Ile Val Lys Arg Asn Asp His Ser Gly Ser Ile Asn Ile Pro
                805                 810                 815

Arg Trp Tyr Ile Thr Ser Asn Leu Gln Asn Tyr Gln Ser Asn Lys Thr
            820                 825                 830

Asp His Lys Ile Glu Gln Leu Ile Gly Lys Asp Gly Ser Tyr Ile Thr
        835                 840                 845

Ser Asp Gln Ile Asp Lys Ile Leu Gln Asp Lys Lys Asp Gly Thr Val
    850                 855                 860

Ile Thr Ser Gln Glu Leu Lys Lys Leu Ala Asp Glu Asn Lys Ser Gln
865                 870                 875                 880

Lys Leu Ser Ala Ser Asp Ile Ala Ser Ser Leu Asn Lys Leu Val Gly
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Ser Met Ala Leu Phe Gly Thr Ala Asn Ser Val Ser Ser Asn Ala Leu
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Leu Val Ile Leu Ala Lys Tyr Tyr Gly Ile Ser Ala Ser Pro Ala Asp
35 40 45

Ile Met His Arg Leu Ala Lys Lys Leu
50 55